SURPLUS FOOD - ITS EFFECT ON PROTEIN METABOLISM WITH OBSERVATIONS ON THE METABOLIC RESPONSE TO INJURY.

By

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PREFACE.

The experimental work which is presented in this thesis, mainly comprises an investigation of the effects of moderate overfeeding on the protein metabolism of the normal and injured human subject. In point of time, the study of the abnormal group preceded that of the normal, but for descriptive purposes the order has been reversed. The experience gained during the investgation of the normal subject was such as would have modified in certain details, the experimental procedure adopted in the study of the effect of forced feeding on the injured subject. Circumstances were such that the further investigation of the latter group could not be pursued.

The main series of experiments on the normal subject owe their existence to the untoward results which were obtained during an investigation, designed to examine certain observations which had come to our knowledge, and which had indicated that a difference existed between the nutritive values of the proteins of raw and boiled milks. The dietary method adopted was of the superimposition type. As the work proceeded, it became obvious that retention phenomena of an unexpected nature were occurring, and were of such an order as to disturb seriously our interpretation of this type of experiment in dietary studies. The investigation was then extended to include a study of the influence of superimposed beef alone, and supplemented with fat and carbohydrate, equivalent in calorie value to that of the milk consumed

in the first series. N retention again took place, being greater when the beef was supplemented with carbohydrate and fat. The investigation then took on a new aspect, and the protein saving effects of carbohydrate and fat, when superimposed on an already adequate diet, were determined. Thus the original intention of this part of the investigation was submerged in an attempt to elucidate these remarkable retention phenomena. In the process some fresh light has been shed on an old chapter of physiology.

In the concluding part of this work there are described some observations on the metabolic response to injury in the rat, and the effect thereon of excess carbohydrate.

INTRODUCTION.

"Protein contains the magic of life, ever newly created and then dying."

Rubner.

Review of the present position of our knowledge concerning the extent of the N retained when excess food is superimposed on a diet adequate for the maintenance of the adult organism.

INTRODUCTION.

The extent of N retention.

The normal fully grown organism tends to establish nitrogen equilibrium by adjusting its rate of protein metabolism to its food supply within wide limits. For practical purposes, we must regard this equilibrium as established when the N intake only exceeds the output in urine and faeces by a few decigrammes. The difference is accounted for by loss in the sweat and from desquamation of epithelial cells, as well as by the small amount required for hair and nail growth. The way in which the body strives to adapt itself to changes in protein intake is well exemplified in the following experiments of Voit (1867) on the dog, and v. Noorden (1907) on man:-

Voit's experiment on the dog.

Date	N intake	N output	Date	N intake	N output
April 13	51.0	51.0	May 31	17.0	18.6
" 14	34.0	39.2	Jan. 1	51.0	41.6
" 15	34.0	36.9	" 2	51.0	44.5
" 16	34.0	37.0	" 3	51.0	47.3
" 17	34.0	36.7	" 4	51.0	49.0
" 18	34.0	34.9	" 5	51.0	49.3
			" 6	51.0	51.0

In the one case the N intake was lowered, in the other raised. What was lost in the first period was gained in the second.

v. Noorden's experiment on man.

	Intake	N in faeces	N in urine	N balance
1st day	14.4	0.70	13.6	+0.1
2nd "	14.4	0.70	13.8	-0.1
3rd "	14.4	0.70	13.6	+0.1
4th "	20.96	0.82	16.8	+3.34
5th "	20.96	0.82	18.2	+1.94
6th "	20.96	0.82	19.5	+0.64
7th "	20.96	0.82	20.0	+0.14

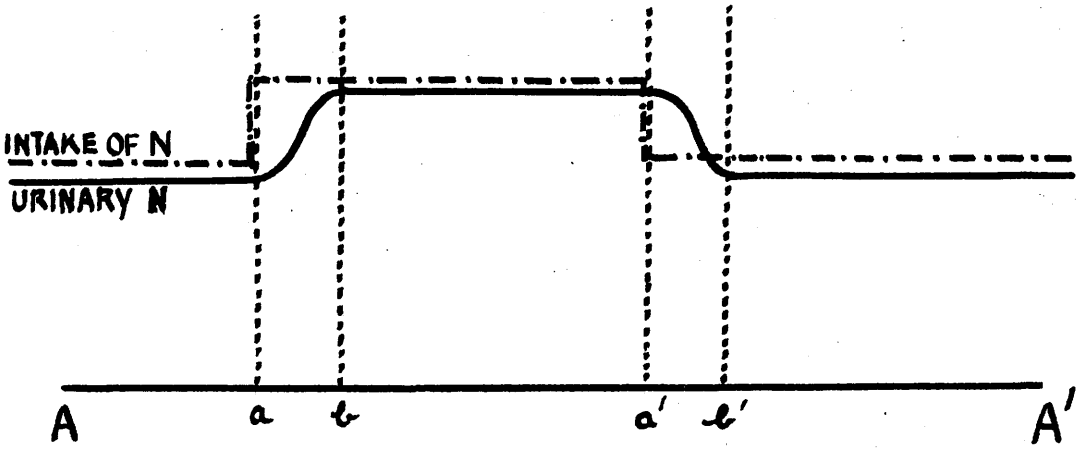
On diminishing the protein intake the opposite occurs, namely, a moderate loss of N.

	N in urine.	N in body.
1st day	18.2	-2.18
2nd "	17.0	-0.98
3rd "	15.8	+0.22
4th "	16.0	+0.02
5th "	15.7	+0.32

In both these experiments the caloric value of the diet was adequate.

If we let the line AA' represent time, the heavy dotted line the intake of protein and the unbroken line the urinary excretion of N, then when the level of protein in the diet

(adequate in calories) is altered the following characteristic graph results:-



N is retained during the interval a-b and lost during the period a'-b'. When the body is accustomed to a certain rate of protein metabolism, there is a time-lag in adjusting itself to a materially higher or lower rate.

Voit (1869) noted that in the dog N equilibrium occurred more rapidly the larger the intake of protein, but if fat were added to an exclusively meat diet, a slight saving of protein was effected for a period. The amount of meat ingested should have been sufficient to establish N equilibrium without extra non-protein calories. The following tables are taken from Voit's article. The first demonstrates little if any saving effect; the second a more definite result.

I.

Date	Intake		Urea	Flesh destroyed.
	Flesh	Fat		
Jan. 19, 1862	1500	0	109.9	
" 20, "	1500	0	110.7	1539
" 21, "	1500	0	109.1	
" 22, "	1500	150	102.0	
" 23, "	1500	150	103.6	
" 24, "	1500	150	107.3	
" 25, "	1500	150	106.1	1473
" 26, "	1500	150	104.1	
" 27, "	1500	150	102.6	
" 28, "	1500	150	109.9	
" 29, "	1500	150	107.6	
" 30, "	1500	150	108.1	1525
" 31, "	1500	150	111.0	

II.

Intake		Urea	Flesh added to body.
Flesh	Fat		
1800	0	127.9	26
1800	0	127.6	26
1800	250	117.9	162
1800	250	113.5	171
1800	250	120.7	171
1800	250	115.7	164
1800	250	119.7	164
1800	250	127.5	11
1800	250	130.0	11

A slight protein saving effect was also noted with carbhydrate. The following table demonstrates this effect in Voit's animal:-

Intake		Urea	Flesh destroyed.
Flesh	Carb.(starch)		
0	0	13.2	181
0	500	10.9	170
500	0	39.2	546
500	250	32.8	475
1500	0	114.9	1599
1500	200	103.3	1454
2000	0	143.7	1991
2000	200	131.1	1825)
2000	200	125.3	1745)
2000	300	124.6	1736) 1792
2000	300	134.3	1868)
2000	300	126.8	1766)

The earlier experiments of Bischoff and Voit (1860) had shown that under conditions leading to fat deposition in the animal, just as little N retention took place as with pure meat feeding. In view of this , subsequent workers have tacitly assumed that in those experiments of Voit's which we have just described, insufficient time had been allowed for the establishment of N equilibrium following the disturbance due to the extra food intake.

The protein retention observed was held to be merely a lag effect. It has generally been considered, that protein storage by the normal adequately fed organism is at best but transitory. In the opinion of Gruber (1901), the temporary retention of protein is simply the result of the superimposition of the hourly curves, and affords an explanation of the apparent retention occurring, when a protein rich diet supersedes a protein poor. The nature of this lag has recently been investigated by Wilson (1931) and by Borsook and Keighley (1935). Wilson's experiment will be described in greater detail later, but it will be convenient here to consider Borsook and Keighley's work. They introduced the term "continuing metabolism" to define the N metabolised on any one day which is already present in the tissues - distinguishing it from exogenous N. It is not related to the endogenous, or "wear and tear" metabolism of Folin. Their experiments were carried out as follows: men were maintained on a constant diet until urinary N and S excretions were constant, then for a 24-hour period the original diet was changed for one in which the N was supplied with little or no S. After equilibrium was re-established with the original diet, there was another interruption, this time a 24-hour period of N starvation but with adequate caloric intake was inserted. The S excreted on the first experimental day was considered a measure of the extent of the continuing metabolism on that day, and, compared with the day of N starvation, indicates the extent to which the continuing metabolism is normally in operation. They consider that in man in N equilibrium at a level of 10-11 g. of urinary N daily, the continuing metabolism constitutes

more than half the total urinary N. The extent of the continuing metabolism appears to be a function of the previous dietary history, and is directly proportional to the level of the N intake.

The observations on protein metabolism described so far have been concerned mainly with the effect of small changes in the level of the protein or non-protein constituents of the diet. Of considerable interest are the results of "forced feeding" when applied to man. Compared with the investigations on the fattening of lower animals, those on man are comparatively few in number. In practically all the recorded experiments the N retentions claimed to have been observed have been made without deduction for cutaneous loss. Further, there has been great dissimilarity in the dietary conditions employed.

It is not our purpose to discuss here the gain in body weight and the accompanying N retention which occurs in those convalescing from wasting disease or previous undernutrition - the results are precisely what would be expected - except in so far as these observations may shed light on the composition of the stored material, or on the general level of metabolism during the realimentation. It is of interest that Hale-White and Spriggs (1899-1900) noted in a woman, organically sound but who had neglected her meals, a retention of 661 g. N and a gain in weight of 13.26 kg. in the course of 56 days. On the basis of I g. N equivalent to 33 g. flesh the expected N retention would have been 402 g., a value far short of that actually found.

One of the first experiments on the normal subject was that carried out by Deiters (1892). The addition of 200 g. sugar

to the diet caused a fall in the N excretion from 10.4 gm. to 9.0 g. and in a second individual from 8.7 g. to 7.5 g. In particular, from v. Noorden's school came several papers indicating that N retention might occur in the normal human subject during forced feeding. During a preparatory period of six days Krug (1893) a healthy individual, attained N equilibrium on a diet of 2590 Calories (44 Cals./kg.). On raising his intake by an additional 1700 non-protein Calories he retained 49.58 g. N in 15 days, the retention being as great at the beginning as at the end. Bornstein (1898) noted a retention of 16 g. N in 14 days when he supplemented a diet sufficient to maintain N equilibrium, with an additional 40 g. protein. Berger (Lüthje and Berger, 1904) retained 32 gm. N during a 7-day period of forced feeding, the diet containing 25-40 g. N and 2700-3300 Calories. On reverting for 5 days to a diet sufficient to cover the body's requirement the N gain was maintained. The observations of Mayer and Dengler (1906) are of interest, in that no increment in the basal consumption of oxygen was noted during a period of forced feeding in a man, who had previously been in a good state of nutrition but was unfortunately not normal at the time of observation. 371 g. N and 13 kg. body weight were gained in 72 days. The N retention was in keeping with the increment in weight. Müller (1911) from the same clinic noted a retention of 210 g. N and an increase of 4 kg. in body weight during 28 days of overfeeding of a normal man. In this case the oxygen consumption rose from 228 to 234 c.c./min., but per kg. body weight, the oxygen intake fell from 4.2 to 4.0 c.c./min. during the period of luxus food consumption. The N retention in this

experiment was greater than that to be expected on the basis of 1 g. N per 33 g. flesh. Mayer and Dengler, and Müller held that the N containing substances added to the body could not have had the same biological properties as the original protoplasm, otherwise there should have been a proportionate rise in the oxygen uptake. These observations have received but little or no attention in recent times.

One of Rubner's most interesting experiments (1903) of which few details have been published, concerned a man who was placed first on a diet containing about 144 g. protein (23.6 g. N), 99 g. fat and 260 g. carbohydrate, and then the fat intake gradually raised until on the fourth day it was some three and a half times the initial value. The N balance on the first day, when the intake equalled 2521 Calories, was -3.64 g. On the final day, when the intake was equivalent to 4676 Calories, the N retention had risen to +5.73 g.

Food				N Metabolism	
N	Fat	Carb.	Total Cal.	N in excreta	N to Body.
23.6	99	260	2521	26.36	-3.64
23.5	195	226	3245	21.55	+1.85
23.0	214	221	3400	18.5	+4.13
23.4	350	234	4676	17.6	+5.75

Rubner (1903) and Bornstein (1904) have also demonstrated that

protein may be retained even when the organism's energy requirement is not met by the food intake. Milk (2438 g.), containing 84 g. protein and two thirds of the requirement of energy for the individual produced a deposit of 6.7 g. N daily for 3 days in Rubner's subject.

In 1910 Grafe first indicated his belief that with increased food intake there is a "Luxuskonsumption" of energy. Long before this however, the striking variations in the total amount of food eaten by different individuals had aroused the idea in the man in the street, that "the person who eats freely is extravagant and that he who eats sparingly can successfully compete with him." Working with Graham (1911) he fed excess food (280 per cent. of the minimal energy requirement) to a 20 kg. dog which had previously been starved for 21 days, until the normal weight and N content were regained (a period of 7 days). This was followed by 29 days of gross overfeeding (300 per cent. of minimal needs), and three subsequent periods with smaller amounts of food but still presumably above the normal requirement. During the latter stages the dog maintained a relatively constant body weight. Grafe reported that during the period of surfeit feeding there was an adjustment of the body's metabolism to the excess nourishment. He based his belief on the fact that after the wasting of the fasting period had been made good, the body weight remained constant, despite continued excess food consumption. In addition, he found that the heat production in the period of excessive feeding, as determined during the "nüchtern" condition, was dis-

tinctly greater than that observed during the starvation period. Benedict et al. (1919) criticised Grafe and Graham's theory of enhanced heat production or "Luxuskonsumption", mainly on the grounds that the heat production during starvation should not have been used as a basis, for the basal metabolism in this state is lower than normal. A further point of contention was that Grafe and Graham assumed that the heat production of the dog outside their respiration chamber (15°) was the same as in the respiration chamber (22°). Benedict et al. recalculated the basal metabolism and held that Grafe and Graham had not positively proved the main point of their discussion. The American workers did point out the striking fact, that during the period of realimentation there was a very great storage of N in the body. In the same year as Benedict and his co-workers published their paper, Eckstein and Grafe published further observations on overfeeding. Table 1 is a synopsis of the first of their experiments on a bitch, in moderately good nutrition with an optimal weight of about 9 kg. In this experiment the fasting period was only 5 days. The surfeit diet consisted of condensed milk, beef and rice. It will be noted that N retention occurred on the various planes of overfeeding, reaching a maximum during the period of greatest intake. During the period of maximum intake the daily retention of N varied considerably, but even at the close of the period it was only slightly less than at the beginning. 79.21 g. N and 3.3 kg. body weight were gained in this period. The maximal weight obtained was not preserved when the diet was decreased to a lower but nevertheless more than adequate plane. These observations

TABLE 1.

Abstract of Eckstein and Grafe's data on bitch "Arett."

Period	Food	Body wt. g.		R.Q.	Calories		Increased per cent over "nüchtern" requirement.	N retention per day.
					24 hrs.	per kg.		
Basal (16 hours p.c.)	Ordinary	7,200		0.853	361	50.2		av. -1.28
Fasting 5th day	Fasting	5,900		0.730	300	50.0		
Considerable over nutrition for 9 days	160 Cal./kg. 5.69 g. N	5,900	1st day	1.072	401	65.8	31.6	av. + 3.18
	(first class)	6,700	last day	1.129	513	75.4	51.0	
Maximal over nutrition for 10 days	1st day 290 Cal./kg. 11.15 g. N	6,800		1.145	666	92.5	82.3	+8.55 av. +6.097
	10th day 210 Cal./kg. 11.15 g. N	9,800		1.171	847	87.3	75.0	+8.02
Considerable over nutrition for 4 days	130 Cal./kg. - 160 Cal./kg.	9,400						
		10,100						+4.56
Moderate over nutrition for 8 days	120 Cal./kg. - 60 Cal./kg. 8.39 g. N	10,240		1.013	734	73.4	49.0	+2.01
		9,700						
Fasting 1st day.		9,700		0.887	506	53.3	6.5	-3.17

were confirmed on other animals. With increasing duration of the overfeeding the organism was found to act more and more uneconomically. The organism apparently gradually adjusted itself by burning more and more of the excess food intake - a process of protection against excessive storage. Eckstein and Grafe removed the thyroid from one of their animals, causing a decrease of 20 per cent. in the metabolic rate. Overnourishment did not now affect the metabolism, which decreased instead of rising with the continuance of the overfeeding. Simultaneously body weight and N retention rose steadily. Subsequent ovariectomy caused a still further reduction in metabolic rate. Again there was no rise in metabolic rate on administering a large quota of food.

In 1923 Grafe replied to Benedict and his co-workers and defended the use of the starvation level as a basis for computation "weil hier allein eine sichere, feste Basis gegehen war, während später gerade unter der Nachwirkung der überreichlichen Ernährung die gewöhnlichen Nüchternwerte anstiegen." On the evidence of his experiments with Redwitz (1922), he held that the temperature difference between cellar and respiration chamber could only account for a 14 per cent. rise in metabolism. The rise in intake was 300 per cent. of the minimal requirement, an increment which would render small errors insignificant. Grafe pointed out that, even admitting Benedict's contention that the excess intake was not of such a large order as appeared in the original calculations, Benedict's recalculated value still indicated that some enhancement of metabolism must have occurred.

Grafe considered his best evidence to be the fact that the oxidative processes, as determined 36-50 hours after the last meal and at a time when the body weight was constant, showed a rise of 40 per cent. over basal.

In his review Grafe indicates that not all persons are capable of "luxus" consumption of energy as is evidenced by the frequent occurrence of obesity. It is common knowledge that men of equal weight and similar constitution, living under apparently similar circumstances, may exhibit marked differences in their food intake. Grafe's work does furnish a nice explanation of the maintenance of a constant body weight and as Du Bois (1928) states, provides a plausible antithesis to the economy of metabolism which accompanies undernutrition.

In support of this theory we have the work of Armsby, Fries and Braman (1918) who found an increase of 36 per cent. in the catabolism of cattle in the fattened state. Helmreich and Wagner (1924) have shown that overnourishment in children leads to an appreciable rise in the basal metabolism, 6-18 per cent. after fat and approximately the same after sugar, and 14-25 per cent. after protein.

The most recent contribution to the vexed problem of "Luxuskonsumption" is the work of Wiley and Newburgh (1931). They criticised the work of Grafe and Graham and pointed out that, although liberal feeding permitted the basal metabolism of their dog to reach a height of 62 per cent. above the starvation level, this was really only 11 per cent. more than the resting and fasting metabolism of the normal dog, prolonged starvation having

reduced it by 26 per cent. Wiley and Newburgh then reinvestigated the problem employing their method of determining heat production by measuring the insensible loss of weight. The subject whom they studied was a very thin tall young man who habitually ate very little, due apparently to indifference to the "substantial joys of eating". Attractive food was set before him, and for a period he overcame his indifference and gained a large amount of weight. During this period neither the basal metabolism nor the total transformation of energy per sq.m. body surface was increased by more than the increment due to the extra specific dynamic action of the additional food. The basal metabolism during the period of maximum intake (5,000 Cals.) was 5 per cent. higher than in the first period (3,000 Cals.) due to this continued specific dynamic action.

It is doubtful however, if the choice of subject for this experiment was appropriate for the purpose of the enquiry, as the authors admit the man's leanness was due to too little food, hence an increment in metabolism was unlikely during the period of high feeding when weight was rapidly being gained. ""Luxuskonsumption" is probably best explained as due to the summated effects of the specific dynamic action of the protein, carbohydrate and fat moieties of the excessive intake." This is the view of Lusk and involves both the "primary" and "secondary" specific dynamic actions described by Rubner. The secondary effect does not affect the fat metabolism, for there is no "secondary dynamic rise" in fat metabolism on a meat diet (Rubner 1902).

Atkinson, Rapport and Lusk (1922) noted that the basal metabolism of a dog on their standard mixed diet was 16 Cal./hr. On the second day after a period of 8 days' meat feeding it was 19.7 and even after two and a half weeks the basal metabolism was still elevated (17.1 Cal./hr.). The work of Kleitmann (1926), Wishart (1928) and Deuel et al.(1928) provides evidence of an enhancement of basal metabolism which accompanies a high protein intake. The correlation between the urinary N and basal metabolism in Wishart's series was remarkably close, and basal metabolic values could be satisfactorily predicted from any given level of nitrogenous metabolism in the author and in another subject studied.

Kriss, Forbes and Miller (1934) in an investigation on rats designed to explain apparent differences between the dynamic effects of food as determined above and below maintenance level, found that the heat increment values for casein, starch and olive oil below maintenance level, when corrected for the sparing of both body protein and fat, agreed reasonably well with the heat increment values of these substances as determined above maintenance.

In their investigation of the diets of families in St. Andrews, Cathcart and Murray (1931) noted that the non-manual workers had a higher calorie intake than the manual. The values for sedentary, semi-manual and manual classes were respectively 3330, 3280 and 3095 Cal./day. They consider that this difference cannot be adequately explained on the basis of a costly expenditure of energy in recreation by the first two classes, or by

undernourishment of the third class. "Luxus" consumption or secondary dynamic action may be a real factor, but the Glasgow workers incline to the opinion that the so-called sedentary, professional or non-manual labouring population is of better physique than the so-called manual labouring population, and consequently the standard metabolism of the sedentary workers will be higher than that of the manual workers. This plus exercise would offer a plausible explanation for the greater calorie value of the food ingested.

Perhaps the most interesting feature of Grafe and Graham's observations, substantiated by later work of Eckstein and Grafe (1919), was the very great storage of N which took place during the realimentation of their starving animals, even after the original optimal weight had been reached. A somewhat similar experiment on the human subject was carried out by Neumann.

These experiments indicate that an individual subsisting on a diet, sufficient to maintain equilibrium and with complete metabolism of the protein of the food, may retain a certain proportion of the food N, if given a larger amount of N-free food material with or without additional protein. If the additional carbohydrate and/or fat be insufficient to cover more than the specific dynamic action of the additional protein, no storage can take place. It is necessary to give large quantities of carbohydrates or fats to save small quantities of protein. According to Voit (1895) not more than 15 per cent. of the previ-

ously metabolised protein can be spared by additions such as the above. On the other hand, even up to 50 per cent. of the total N excreted during complete starvation may be spared by giving a free supply of carbohydrate. In Bonn, in the winter of 1916/17, Neumann conducted a very interesting investigation on himself which has received but scant attention (Table 2). On a diet containing 1000 g. rye bread, N though not weight equilibrium was attained. The calorie value of this diet was 2425 (42.3/kg.). The fall in body weight was arrested when 300 g. cane sugar were added, and a retention of N with increase in body weight soon took place. On raising the cane sugar intake by an additional 200 g. (total Cal. 4434) further increase in weight and N retention took place, the N retention tending to reach a constant daily value. Pediatricians have long recognised that the marked weight increase which occurs in children fed excess carbohydrate, is accompanied by a marked retention of water. The daily N excretion of Neumann during the period of N equilibrium was 10.48 g. (urinary N, 8.04 g.: faecal N, 2.44 g.) and at the close of twenty three days' excessive feeding had fallen to 7.30 g. (urinary N, 4.89 g.; faecal N, 2.41 g.). There was no evidence that N equilibrium would again be attained. From the point of view of the present discussion, it is perhaps unfortunate that these periods of excess feeding had been preceded by intervals of relative starvation. Despite this defect the experiments illustrate the sensitiveness of the N balance to the addition of carbohydrate.

TABLE 2.

The average daily metabolic data derived from Neumann's personal experiment during the winter of 1916/17, when he took as his sole food varying amounts of "Vollkornbrot" supplemented in the later periods with sugar.

Period	Days	Protein intake (g.N)	Fat g.	Carb. g.	Total Cal.	Cal/Kg	Total N output. g.	N Balance g.
1	6	31.0 (4.95)	2.75	254	1212	20.6	13.10	-8.15
2	6	46.5 (7.43)	4.13	381	1818	31.4	12.60	-5.17
3	5	62.0 (9.90)	5.5	508	2425	42.3	11.89	-1.99
4	5	62.0 (9.90)	5.5	802	3630	63.8	10.48	-0.58
5	8	62.0 (9.90)	5.5	998	4434	77.1	8.30	+1.60
6	5	62.0 (9.90)	5.5	998	4434	74.5	7.62	+2.28
7	5	62.0 (9.90)	5.5	998	4434	72.3	7.30	+2.60

This brief review of the early literature has brought to light many observations which indicate that N retention may result from overfeeding the normal organism. Little cognisance of these observations has been taken and it is rare to find reference to them in the current literature. So far, no reference has been made to the nature and site of the retained N. Discussion of this most interesting aspect of the problem is delayed meantime. At this point it will be more convenient to describe certain experiments which we made to determine:

(1) The effect of superimposing raw and boiled milks on a diet adequate for maintenance.

(2) The effect of superimposing on a diet adequate for maintenance, beef (or soya flour) + lactose + butter, equivalent in protein, carbohydrate and fat content to a litre of milk.

In the prosecution of this investigation I have been greatly aided by Mr H. M. Munro, an honours student in this Department, and by Mr A. McCutcheon, laboratory attendant. I am also greatly indebted to Messrs Chambers, Gibson, Green, Orchardson and Stirrat for their loyal support in acting as experimental subjects.

PART 1.

THE EFFECT OF SUPERIMPOSING RAW AND BOILED MILKS ON A DIET ADEQUATE FOR MAINTENANCE.

EXPERIMENTAL.

The subjects selected for these experiments were students and members of the teaching staff performing routine laboratory duties. They were all of good physique. During the experimental period they took no special exercise apart from short walks of a relatively uniform character.

Urine was collected in 24-hour specimens, made up to constant volume and preserved with thymol in chloroform. The various periods during which faeces were collected were marked off with carmine, taken in the form of capsules. The faeces were acidified with H_2SO_4 and dried on a steam bath and in an air-oven before being stored in sealed bottles. If S is also being determined the technique must be modified, by adding to the fresh faeces a dilute solution of copper acetate before drying them in a closed vessel through which a current of dry ammonia-free air passes. The ammonia liberated from the faeces is trapped in acid.

The following experiments indicate the extent of the nitrogen and sulphur loss during drying. Total N was estimated by the Kjeldahl method; total S by Denis's modification of Benedict's ashing method. Analyses were performed in triplicate, first on the wet specimens and then on aliquot portions subjected to various procedures. The accuracy of the sampling of these wet specimens was confirmed by the close agreement of triplicate estimations.

All specimens of human faeces were analysed within an hour or two of being voided.

Exp. 1. Sample of faeces containing 0.8976 g. total N had after 10 days' continuous drying on a steam-bath 0.8439 g. N. % loss = 5.9.

Exp. 2. Sample of faeces containing 0.616 g. N had after 14 days' drying on a steam-bath 0.558 g. N. % loss = 9.4.

Exp. 3. Sample of faeces containing 1.599 g. N and 0.222 g. S contained after 14 days' continuous drying on a steam-bath 1.531 g. N and 0.116 g. S. % loss N = 4.2. % loss S = 47.

Exp. 4. Fresh wet faeces containing 1.305 g. N were transferred to a vacuum-pan and the temperature of the bath raised to 80°. Ammonia-free air was passed over the faeces and drying was carried out under reduced pressure. In the course of 1 hour during which the temperature of the bath was rising no ammonia came over. In the next 5 hours 0.0286 g. N was trapped in N/10 H_2SO_4. That the loss of N was as NH_3 was confirmed by Nesslerisation. During the subsequent 6 hours only 0.0007 g. more N came over. Loss of N = 2.2% in course of 14 hours' drying.

In an attempt to prevent the simultaneous loss of N and S from faeces by means of a preliminary treatment with substances which would trap NH_3 and H_2S no simple method could be devised. It was eventually determined that the most satisfactory method was to moisten the faeces with excess 2% copper acetate and trap the ammonia in acid during the process of drying.

Exp. 5. Sample of faeces containing 0.114 g. S contained after drying with copper acetate 0.115 g. S. There was thus no loss of S. When an aliquot portion was dried without any precaution for a similar period, viz. 2 days, the dried faeces contained

0.109 g. - a loss of 4.8% S.

From clinical and bacteriological considerations it is obvious that the amounts of free gases contained in faeces must vary considerably.

Exp. 6. A sample of faeces containing 1.867 g. N was dried in the presence of 2% copper acetate for 24 hours. Ammonia-free air was passed over the faeces and then through standard H_2SO_4. Ammonia was estimated by Nesslerisation as it was found that acetic acid was also driven over. The total amount of trapped N was 0.275 g. The total residual N was 1.591 g. 1.866 g. N of the original 1.867 g. was thus accounted for.

The original sample of faeces contained 0.1705 g. S. An aliquot dried without precaution for 24 hours contained 0.147 g. S and one dried for 6 days contained 0.1355 g. S - losses of 14% and 20% respectively. An aliquot treated with copper acetate lost no S on drying for 24 hours.

Exp. 7. A sample of faeces containing 2.522 g. N was dried in presence of 2% copper acetate for 48 hours. Ammonia-free air was passed over the faeces and through a 10% NaOH trap to absorb the acetic and other volatile acids. The air was finally passed through standard acid. In the final estimation the residual ammonia was distilled over from the NaOH solution and added to that trapped in the acid. The total loss was 0.0339 g. N. The nitrogen content of the dried faeces was 2.495 g. The original 2.522 g. were thus accounted for.

The original sample of faeces contained 0.588 g. S. An

aliquot dried without precaution for 24 hours contained 0.581 g. S and one dried for 5 days 0.5675 g. S - losses of 1.2% and 3.5% respectively. An aliquot dried with 2% copper acetate lost no S on drying for 24 hours.

In these experiments allowance was made for the trace of S in the copper acetate by adding aliquot amounts at the appropriate times. It was thus added to ordinary air-dried specimens of faeces after the process of drying was complete but before analysis

It is seen from these experiments that an accurate analysis of the nitrogen and sulphur of faeces is possible if in the process of drying, ammonia-free air is passed over the faeces to which have been added a dilute solution of copper acetate in excess, the air is then passed through alkali to trap acetic and other volatile acids and then through standard acid. The alkaline fluid traps some ammonia and this is finally distilled into the excess standard acid.

Analytical Methods.

(a) Urine:

Total nitrogen	-	Kjeldahl method.
Urea -	-	Urease method using Maclean's blood urea apparatus and 5 ml. urine.
Ammonia -	-	Aeration method using same apparatus and 20 c.c. urine.
Creatinine -	-	Folin's method.
Total sulphur	-	Denis's modification of Benedict's method.
Inorganic sulphur	-	Folin's method.
Calcium -	-	Shohl and Pedley's method.

(b) Faeces and food:

Total nitrogen	-	Kjeldahl method.
Total sulphur	-	Denis's modification of Benedict's method.
Calcium	-	Material ashed, any iron removed, then precipitated according to Shohl and Pedley's method.

The effect of superimposing raw and boiled milk on a diet adequate for maintenance.

Experimental series:

1. One day superimposition experiments.

2. Eight day superimposition experiments.

First series - one day superimposition experiments.

Eight healthy male subjects of good physique ranging in age from 19-35 years were placed on self-selected constant basal diets comprised of white and brown breads, 100 g. fresh butter, cheese, 500 ml. raw milk (Grade A. T.T.), apple jelly and raw apples. The water intake was kept constant. Subjects 5, 6, and 7 received lettuce and tomato in addition. The diet was consumed four meals per day.

The calorie intake varied from 34 to 52 with a mean of 43 Cal./kg./ day gross, a value approximately equal to the calculated requirement. The Technical Commission appointed by the Health Committee of the League of Nations (1935, 1936) defined the calorie requirement of an adult living an ordinary every-day life in a temperate climate and not engaged in manual work to be 2,400 Cal. nett. To this must be added the supplement necessary for light or moderate work. The additional supplements required in these experiments would be in the region of six hours 'light work' at 45 Cal. per hour, and one to two hours of 'moderate work' at 65 Cal. per hour, making a total requirement of about 2,800 Cal. nett, equivalent to 40 Cal./kg./day.

The protein intake varied from 0.82-1.21 g./kg. with a mean of 1.02 g., a value equal to the minimum practicable standard advocated by Sherman and thought by the League of Nations Committee (1935) to be sufficient. Of this protein 25-52% was 'first class'. This level of total protein was chosen in order that the nutritive properties of the superimposed protein might be more readily brought to light without rendering the

diets abnormal. The variations in protein intake among the members of the group are mainly due to differences in the consumption of cheese. The intake of this foodstuff varied from 0 for Subject 7 to 90 g. per day for Subject 3.

TABLE 1.

Subject	Age	Initial Body wt. kg.	Final Body wt. kg.	Cal./kg.	Prot./kg.	% First class protein.
(1) Gi	19	66.75	66.70	45	1.09	51
(2) Gr	20	74.3	74.70	37	0.82	41
(3) Or	19	56.6	56.90	51	1.21	54
(4) St	21	74.9	74.80	39	1.01	52
(5) Cu	35	87.0	86.70	34	0.92	33
(6) Mu	20	60.2	60.25	45	1.16	30
(7) McC	29	56.1	57.0	52	0.87	25
(8) Ch	25	64.6	64.8	42	1.08	30

With the exception of Subject 8 a fairly steady urinary excretion was observed on the basal diets. Although no determinations of the faecal N excretion were made in this series the individuals were probably in N equilibrium on these diets. This is supported by the fact that the body weights tended to remain constant during the experimental periods which lasted 3-5 weeks in length. Subject 7 was the only person to show a definite increase in weight during the period (0.9 kg. in 4 weeks). This was due to the higher calorie diet selected.

Subject 8, a member of the University teaching staff, ex-

Day	Body weight, kg.	Urinary volume, ml.	Urinary N, g.
1	64.9	1,660	14.26
2	64.8	1,108	12.71
3	65.2	1,077	11.41
4	65.2	1,318	13.82
5	65.3	1,000	11.60
6	[illegible]	1,088	12.28
7	65.2	1,810	13.68
8	65.4	1,408	12.58
9	65.7	1,097	12.32
10	65.85	1,088	12.10
11	65.4	1,078	[illegible]
12	[illegible]	1,740	[illegible]
13	64.4	[illegible]	[illegible]
14	65.2	1,470	12.12
15	64.65	1,760	11.44
16	65.0	2,181	11.42
17	65.2	1,720	[illegible]
18	65.15	1,860	12.10
19	64.80	1,870	11.3
20	-	1,802	11.1

Urine 1 day
of mon milk - 6

TABLE 2.

Subject 8.

Day	Body Weight kg.	Urinary volume ml.	Urinary T.N. g.
1	64.6	1,595	10.28
2	64.5	2,005	10.72
3	65.2	1,277	8.76
4	65.2	1,315	9.21
5	65.3	1,000	9.38
Extra litre of raw milk - 6 for 1 day	65.0	1,355	12.29
7	65.2	1,510	9.85
8	65.2	1,408	9.68
9	65.1	1,087	10.12
10	65.25	1,025	11.19
11	65.4	1,675	10.80
12	65.0	1,740	10.19
13	64.8	2,285	9.85
14	65.2	1,078	10.19
15	64.85	1,760	11.94
16	65.0	2,055	10.42
17	65.2	1,190	9.97
18	65.15	1,840	11.00
19	64.80	1,670	9.72
20	-	1,302	9.84

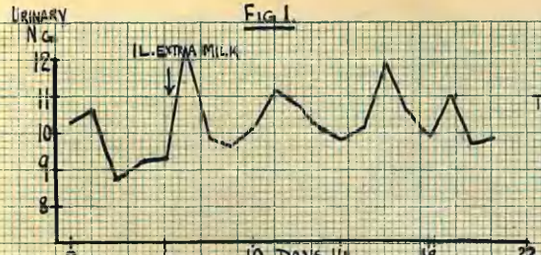


Fig 1

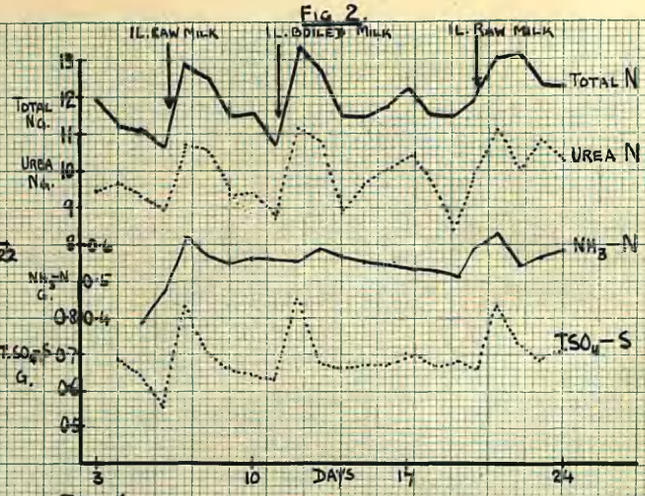


Fig 2.

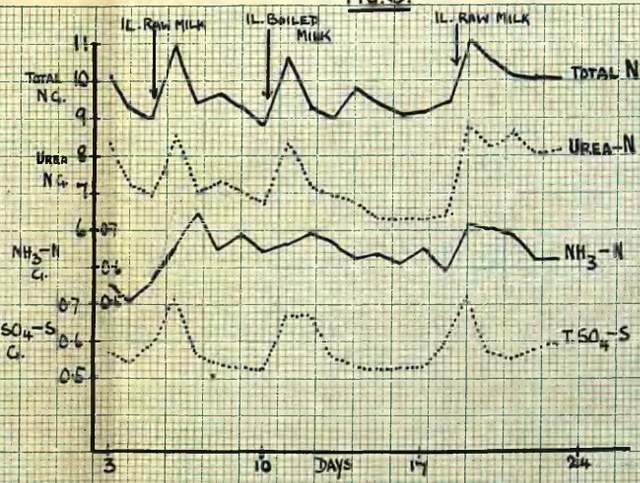
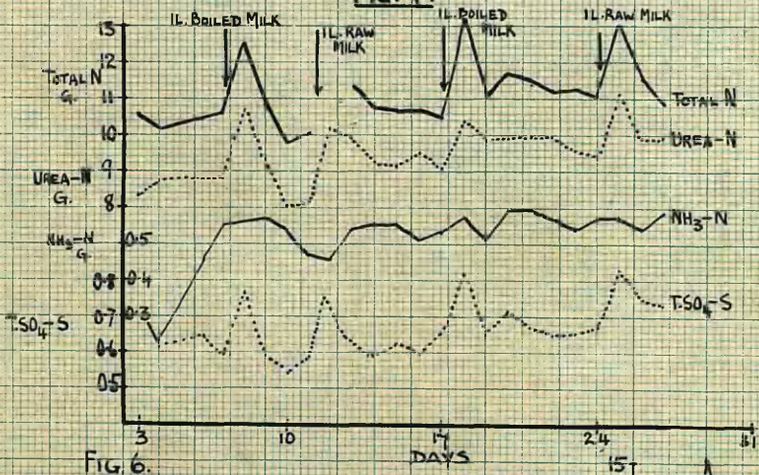


Fig. 3.



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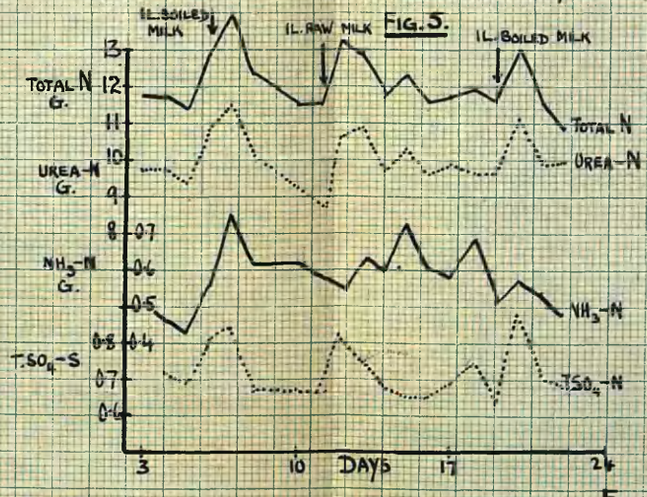

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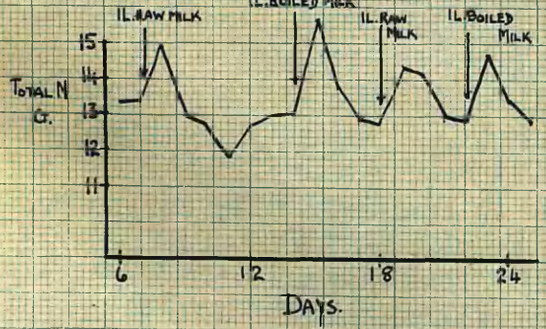


Fig. 6.

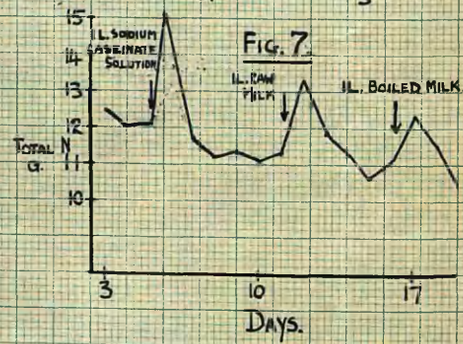


Fig. 7.

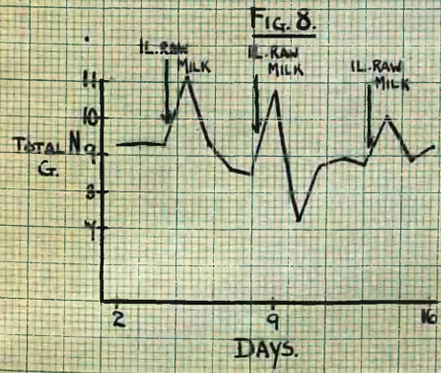


Fig. 8.

hibited a very irregular daily excretion of N when on basal diet despite the most rigid control of his daily routine. This irregularity which was quite unaccountable was so great that the experiment had to be abandoned. There was nothing to account for this variability. The subject's daily N excretion is recorded in Table 2 and Fig. 1.

Having established equilibrium Subjects 1-7 received on one day an extra litre of milk, the extra intake of water in the milk being compensated by a withdrawal of an equivalent volume of drinking water. As soon as N equilibrium was re-established the addition of extra milk was repeated. The order of administration is indicated in Table 3, the extra milk being consumed in two lots of 500 ml. each at 1 p.m. and 5 p.m.

TABLE 3.

Subject	Period 1	Period 2	Period 3	Period 4
1	Raw	Boiled	Raw	-
2	Raw	Boiled	Raw	-
3	Boiled	Boiled	Raw	-
4	Boiled	Raw	Boiled	-
5	Raw	Boiled	Raw	Boiled
6	Raw	Boiled	-	-
7	Raw	Raw	Raw	-

It may be noted here that even when boiled milk was superimposed on the basal diet the latter contained its usual quota of raw milk. This constancy of basal was maintained so that if any differences were subsequently apparent in the fate of the N of the milk, they could definitely be ascribed to a change in the protein caused by boiling, and not to some unknown deficiency in the diet which might be presumed to be due to the absence of raw milk.

During the month of May when Subjects 1-4 were on diet the average N content of a litre of Grade A. T.T. milk was 5.58 g. and during July when Subjects 5-7 were on diet, 5.63 g. In boiling the milk a cast aluminium pan was used, the milk being stirred constantly till the boiling point was just reached. It was then cooled. The skin and residue which stuck to the sides and bottom of the pan were consumed.

In Table 4 are recorded the amounts of extra N excreted by the different individuals on the day of ingestion of the extra milk and the day following. The mean values for the extra urinary N excreted by Subjects 1-6 on the first day were, for raw milk, 1.683 g.; for boiled milk, 1.980 g.: for the second day, 0.889 g. raw and 0.697 g. boiled milk. The mean values for the total extra N excretion during the two days were 2.572 g. and 2.677 g. for raw and boiled milks respectively, a difference of 4%. These differences are not statistically significant (Table 10). Tables 5-9 and Figs. 2-8 show the day-to-day variation in the excretion of total N, urea-N, NH_3-N, total sulphates-S and urinary volume. It will be appreciated that urea parallels the total N

TABLE 4.

Subject	Calories	Basal N (urine) g.				Extra milk N per litre g.	Type of milk	Rise of N over basal (urine) g.			Rise of S over basal (urine) g.
		1.	2.	3.	4.			1st day	2nd day	Total	1st day
1	2,932	11.02	11.27	11.69	-	Av. 5.58	Raw Boiled Raw	1.85 2.03 1.32	1.52 1.50 1.45	3.37 3.53 2.77	0.205 0.202 0.155
2	2,674	9.18	9.06	9.33	-	"	Raw Boiled Raw	1.74 1.54 1.75	0.26 0.17 1.22	2.00 1.71 2.97	0.137 0.140 0.166
3	2,909	10.45	-	10.70	11.18	"	Boiled Boiled Raw	2.05 2.45 1.82	0.47 0.34 0.36	2.52 2.79 2.18	0.151 Raw 0.181 0.200 0.169
4	2,976	11.77	11.54	11.73	-	"	Boiled Raw Boiled	2.16 1.67 2.14	0.61 1.38 1.87	2.77 3.05 4.01	0.138 0.161 0.179
5	2,959	13.39	13.03	12.85	12.94	5.63	Raw Boiled Raw Boiled	1.51 2.61 1.53 1.81	0.00 0.66 1.26 0.46	1.51 3.27 2.79 2.27	- - - -
6	2,698	11.29	11.29	-	-	"	Raw Boiled	1.96 1.03	0.54 0.19	2.50 1.22	- -
7	2,907	9.28	8.61	8.80	-	"	Raw Raw Raw	1.84 2.21 1.25	- - -	- - -	- - -

TABLE 5.

The effect of the superimposition of 1000 ml. raw and boiled milk (≡ 5.58 g.N) on an adequate basal diet.

SUBJECT 1.

Urine(g)

Diet	T.N.	Urea -N	NH_3 -N	SO_4 -S
Basal (3rd day)	11.98	9.48	0.244	-
"	11.25	9.735	0.265	0.690
"	11.18	9.34	0.386	0.644
"	10.63	8.98	0.468	0.557
+ 1 L. raw milk	12.87	10.79	0.619	0.835
Basal	12.54	10.53	0.571	0.702
"	11.49	9.37	0.551	0.662
"	11.57	9.43	0.563	0.655
"	10.75	8.75	0.567	0.629
+ 1 L. boiled milk	13.30	11.16	0.557	0.850
Basal	12.77	10.80	0.588	0.682
"	11.58	8.81	0.576	0.665
"	11.47	9.65	0.554	0.671
"	11.75	10.00	0.543	0.672
"	12.23	10.48	0.536	0.702
"	11.47	9.73	0.522	0.668
"	11.43	8.41	0.512	0.683
"	11.91	9.93	0.595	0.673
+ 1 L. raw milk	13.01	11.09	0.629	0.831
Basal	13.14	10.03	0.542	0.725
"	12.35	10.82	0.568	0.684
"	12.12	10.20	0.584	0.717

TABLE 6.

The effect of the superimposition of 1000 ml. raw and boiled milk (≡ 5.58 g.N) on an adequate basal diet.

SUBJECT 2.

Urine (g).

Diet	T.N.	Urea -N	NH_3 -N	SO_4 -S
Basal (3rd day)	10.09	8.31	0.548	0.572
"	9.32	7.20	0.512	0.542
"	9.04	6.97	0.560	0.588
+ 1 L. raw milk	10.92	8.585	0.655	0.705
Basal	9.44	7.05	0.741	0.566
"	9.60	7.30	0.648	0.539
"	9.27	7.03	0.691	0.531
"	8.85	6.725	0.645	0.523
+ 1 L. boiled milk	10.595	8.29	0.662	0.671
Basal	9.23	7.17	0.692	0.671
"	9.00	6.95	0.675	0.557
"	9.83	7.76	0.624	0.538
"	9.41	7.37	0.638	0.522
"	9.12	7.27	0.613	0.527
"	9.19	7.37	0.651	0.531
"	9.44	7.42	0.598	0.598
+ 1 L. raw milk	11.08	8.78	0.715	0.721
Basal	10.55	8.22	0.700	0.572
"	10.12	8.70	0.684	0.553
"	10.06	8.05	0.624	0.571
"	10.05	8.06	0.620	0.599

TABLE 7.

The effect of the superimposition of a 1000 ml. raw and boiled milk (≡ 5.58 g.N) on an adequate basal diet.

SUBJECT 3.

Diet	Urine (g) T.N.	Urea -N	NH_3 -N	SO_4 -S
Basal (3rd day)	10.59	8.38	0.302	-
"	10.18	8.79	0.224	0.613
"	-	-	-	-
"	10.43	8.82	0.462	0.637
"	10.61	8.80	0.533	0.592
+ 1 L. boiled milk	12.50	10.68	0.556	0.764
"	10.92	9.29	0.567	0.594
"	9.79	8.05	0.540	0.544
"	10.00	8.16	0.466	0.588
+ 1 L. raw milk	11.52*	10.24	0.462	0.746
"	11.37	9.90	0.543	0.640
"	10.78	9.25	0.550	0.596
"	10.75	9.16	0.554	0.622
"	10.72	9.55	0.512	0.599
"	10.55	9.02	0.529	0.648
+ 1 L. boiled milk	13.15	10.41	0.577	0.816
"	11.04	9.88	0.505	0.654
"	11.69	9.89	0.591	0.715
"	11.51	9.99	0.598	0.671
"	11.23	9.93	0.573	0.654
"	11.26	9.56	0.540	0.656
"	11.06	9.37	0.573	0.671
+ 1 L. raw milk	13.00	11.11	0.570	0.829
"	11.54	9.86	0.541	0.649
"	10.89	9.96	0.483	0.633

* Analysis could not be repeated as urine accidentally lost. T.N. unsatisfactory so figure not incorporated in final computation.

TABLE 8.

The effect of the superimposition of 1000 ml. raw and boiled milk (≡ 5.58 g.N) on an adequate basal diet.

SUBJECT 4.

Diet	Urine (g)			
	T.N.	Urea -N	NH_3 -N	SO_4 -S
Basal (3rd day)	11.76	9.78	0.501	-
"	11.77	9.81	0.470	0.721
"	11.39	9.40	0.432	0.692
"	12.92	10.82	0.560	0.804
+ 1 L. boiled milk	13.93	11.46	0.748	0.844
Basal	12.38	10.14	0.608	0.661
"	-	-	-	-
"	11.51	9.22	0.620	0.661
"	11.57	8.72	0.579	0.659
+ 1 L. raw milk	13.21	10.62	0.544	0.821
Basal	12.92	10.91	0.634	0.746
"	11.70	9.76	0.605	0.681
"	12.31	10.27	0.718	0.677
"	11.61	9.59	0.601	0.652
"	11.77	9.90	0.584	0.698
"	11.92	9.81	0.685	0.745
"	11.63	9.61	0.519	0.736
+ 1 L. boiled milk	13.00	11.11	0.570	0.880
Basal	11.54	9.86	0.541	0.718
"	10.89	9.96	0.483	0.669

TABLE 9.

Subjects 5, 6 and 7.

Subject 5		Subject 6		Subject 7	
Diet.	T.N.	Diet	T.N.	Diet	T.N.
Basal	13.38	Basal	12.45	Basal	9.24
"	13.41	"	12.01	"	9.28
+ 1 L.raw milk (≡ 5.63 g.N)	14.90 (25 hrs.)	"	12.15	"	9.24
Basal	12.97 (23 hrs.)	+ 1 L.sodium caseinate soln. (≡ 5.7 g.N)	15.09	+ 1 L.raw milk (≡5.63 g.N)	11.12
"	12.82	Basal	11.70	Basal	9.28
"	11.87	"	11.24	"	8.65
"	12.67	"	11.37	"	8.57
"	13.00	"	11.09	+ 1 L. raw milk (≡5.63 g.N)	10.81
"	13.06	"	11.29	Basal	7.20
+ 1 L.boiled milk (≡ 5.63 g.N)	15.635 (25 hrs.)	+ 1 L.raw milk (≡ 5.63 g.N)	13.25	"	8.72
Basal	13.69 (23 hrs.)	Basal	11.83	"	8.89
"	12.89	"	11.33	"	8.79
"	12.81	"	10.64	+ 1 L. raw milk (≡ 5.63 g.N)	10.05
+ 1 L.raw milk (≡ 5.63 g.N)	14.38	"	11.09	Basal	8.86
Basal	14.11	+ 1 L.boiled milk (≡ 5.63 g.N)	12.32	"	9.20
"	12.99	Basal	11.48		
"	12.89	"	10.40		
+ 1 L. boiled milk (5.63 g. N)	14.75 13.40				
"	12.80				

TABLE 10.

Statistical summary of data on Table 4.

	N						S	
	1st day		2nd day		Total two days		1st day	
	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
Mean	1.683	1.980	0.889	0.697	2.571	2.677	0.168	0.168
S.D.	0.1923	0.4494			0.5495	0.8228		

excretion whereas ammonia exhibits a steady rate of elimination. Total sulphates parallel the total N and urea-N output but the excess output appears to be all excreted on the first day, indicating a more rapid elimination of S, a fact in harmony with most work on N and S metabolism. As early as 1881, Feder had noted that the S of ingested protein was excreted earlier than the N. Rubner (1902) and von Wendt (1905) confirmed the more rapid elimination of S following meat ingestion. Wolf and Osterberg (1911) found a lag in the excretion of S of superimposed egg-white, and Lewis (1916) found, on feeding starved dogs with meat, a lag in the S output. Sherman and Hawk (1901), on the other hand, have shown an almost parallel excretion of S and N. Cathcart and Burnett (1926) found that with moderately severe work the S rose pari passu with the output of N. The accumulated evidence seems to indicate that it is the nutritive state of the organism at the time which determines the fate of the components of the ingested protein, but that normally there is a delay (Wilson, 1931). The mean of the excess S excretions over basal was 0.168 g. S for both

TABLE 10.

Statistical summary of data on Table 4.

	N						S	
	1st day		2nd day		Total two days		1st day	
	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
Mean	1.683	1.980	0.889	0.697	2.571	2.677	0.168	0.168
S.D.	0.1923	0.4494			0.5495	0.8228		

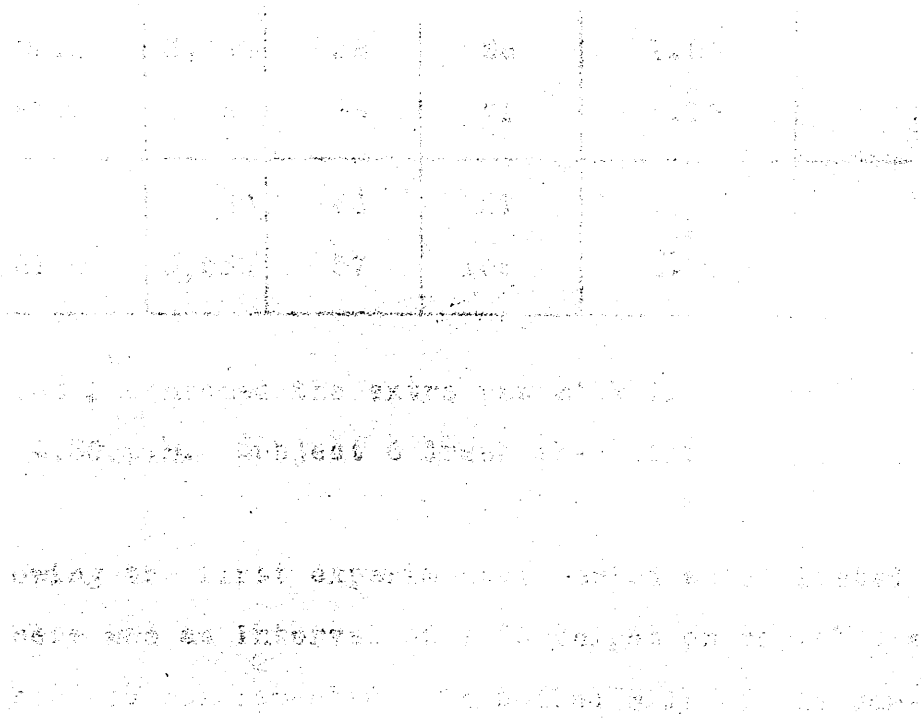
excretion whereas ammonia exhibits a steady rate of elimination. Total sulphates parallel the total N and urea-N output but the excess output appears to be all excreted on the first day, indicating a more rapid elimination of S, a fact in harmony with most work on N and S metabolism. As early as 1881, Feder had noted that the S of ingested protein was excreted earlier than the N. Rubner (1902) and von Wendt (1905) confirmed the more rapid elimination of S following meat ingestion. Wolf and Osterberg (1911) found a lag in the excretion of S of superimposed egg-white, and Lewis (1916) found, on feeding starved dogs with meat, a lag in the S output. Sherman and Hawk (1901), on the other hand, have shown an almost parallel excretion of S and N. Cathcart and Burnett (1926) found that with moderately severe work the S rose pari passu with the output of N. The accumulated evidence seems to indicate that it is the nutritive state of the organism at the time which determines the fate of the components of the ingested protein, but that normally there is a delay (Wilson, 1931). The mean of the excess S excretions over basal was 0.168 g. S for both

raw and boiled milk. This emphasises the fact that no significant difference in the quality of raw and boiled milk proteins could be detected under these experimental conditions. The variability in the excess N excretions in these experiments is due to the fact that the individual's reaction to a superimposed protein is never quite the same each time. This was confirmed by noting the reaction of Subject 7 to the ingestion of 1 litre raw milk on three separate occasions, N equilibrium having been established in the intervals. The excess N outputs were 1.84 g., 2.21 g. and 1.25 g.

The degree of retention observed did not appear to bear any direct relationship to the amount of first class protein in the diet which was governed in these diets by the subject's like or dislike of cheese; the only other source of first class protein, the milk intake, being kept constant. The mean percentage of the extra milk N was 54%, that of S, 53%. The material retained during these raw and boiled milk experiments had apparently an S:N ratio of 1:13.4 and 1:13.9 respectively. Since the extra milk ingested had an S:N ratio of 1:15.5 this would indicate that the material stored was richer in S than that ingested. The S:N ratio is close to that obtaining in muscle. As will be pointed out later, the S:N ratio of the material retained on the first day of a superimposition experiment need not be taken as an index of the value of the ratio during a more prolonged period, and therefore it appears unwise to stress the significance of these figures.

To test if the capacity of the organism to retain milk N was equal to or greater than its capacity to store the N of an

equivalent amount of sodium caseinate, Subject 6 ingested a litre of sodium caseinate in place of a litre of milk. Only 33% of the caseinate N was retained as compared with retentions of 56% and 69% noted with raw and boiled milks (Fig. 7). It appeared as if the non-protein moiety of the milk contributed to the storage of the N. The question naturally arose as to whether this extraordinary retention would still occur when the period of milk ingestion was prolonged. For this purpose a second series of experiments were designed.



Second series - Eight day superimposition experiments.

Subjects 5 and 6 took part in this series. The basal diets were identical in quality and only slightly different in quantity to those consumed in the first series and were consumed in the winter of 1935-36. The ingestion of the extra litre of milk increased the total caloric value and protein content of the diets by some 680 Cal. and by 35 g. protein (first class). It was continued for a period of 8 days in each instance.

TABLE 11.

Diet	Subject	Initial body wt.	Total Cal.	Cal./kg.	Total protein g.	Protein/kg. g.	Per Cent. First class protein
Basal	5	86.2	3,300	38	86	1.00	31
"	6	61.5	2,850	46	71	1.15	30
Basal + 1,000 ml. milk	5	86.2	3,980	46	121	1.40	51
	6	61.5	3,530	57	106	1.72	53

Subject 5 consumed the extra raw milk in two equal lots at 1 p.m. and at 4.30 p.m. Subject 6 drank the litre of milk at 9.15 a.m.

Following the first experimental period which lasted about three weeks there was an interval of a fortnight on normal diet before the procedure was repeated with boiled milk of the same quality as the raw.

It will be noted that the urinary N excretion exhibits the same general trend in the same subject irrespective of the treatment of the milk (Figs. 9 and 10, Tables 12 and 13). The two subjects behaved rather differently to this extra milk N. In the case of Sub-

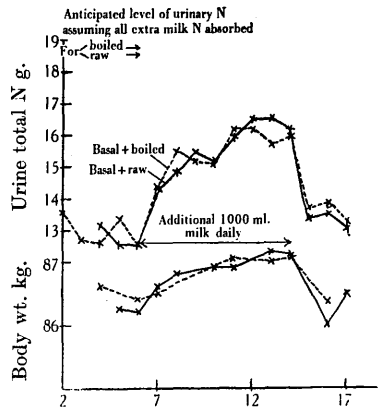


Fig. 9 Subject 5. Days of experiment

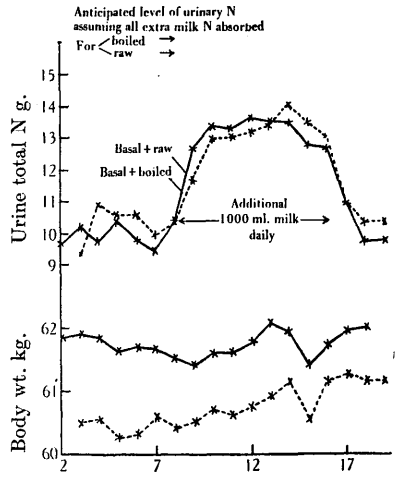


Fig. 10 Subject 6. Days of experiment

TABLE 12.

Subject 5.

Eight days' superimposition of raw and boiled milks.

Diet	Body weight kg.	Daily N intake g.	Urine vol. ml.	N output daily g. Urine	Faeces	Total
Basal 3,300 Cal.	86.25 86.20	14.85	2,260 2,185 1,880	13.15 12.57 12.47		
			Mean	12.73	1.967	14.70
Basal + 1 litre raw milk 3,980 Cal.	86.60 86.80 86.90 86.90 87.15 87.10	20.63	1,476 1,605 1,548 1,365 1,760 2,100 1,755 2,065	14.23 14.81 15.42 15.18 15.92 16.45 16.46 16.15	1.963	
			Total 8 days	124.62		140.32
Basal 3,300 Cal.	86.60 86.00 86.50	14.85	1,955 1,480 1,510	13.36 13.47 13.05		
			Total 11 days	164.50		
Basal 3,300 Cal.	85.90 86.60 86.40	14.97	2,015 1,755 1,860 1,930 2,070	13.55 12.66 12.59 13.33 12.48	1.967	
			Mean	12.92		
Basal + 1 litre boiled milk 3,980 Cal.	86.50 87.05 87.0 87.05	20.78	1,375 2,095 1,870 1,830 1,705 1,900 2,140 1,705	14.37 15.43 15.19 15.07 16.17 16.15 15.66 15.95	2.396	
			Total 8 days	123.99		143.16
Basal 3,300 Cal.	86.35	14.97	1,870 1,775 1,700	13.64 13.84 13.22		
			Total 11 days	164.69		

TABLE 13.

Subject 6.

Eight days' superimposition of raw and boiled milks.

Diet	Body weight kg.	Daily N intake g.	Urine vol. ml.	N output daily g.		
				Urine	Faeces	Total
Basal 2,850 Cal.	61.85	12.02	1,520			
	61.90		1,465	9.20		
	61.85		2,023	9.64		
	61.60		1,982	10.21		
	61.70		1,652	9.75		
	61.65		1,996	10.38		
	61.50		2,216	9.79		
			1.997	9.41		
				10.38		
			Mean	9.85	1.21	11.06
Basal + 1 litre raw milk 3,530 Cal.	61.40	17.80	1,384	12.64		
	61.60		1,397	13.19		
	61.60		1,667	13.24		
	61.75		1,450	13.62	1.591	
	62.05		1,875	13.55		
	61.90		1,748	13.51		
	61.40		990	12.79		
	61.75		1,810	12.68		
			Total 8 days	105.22	12.73	117.95
Basal 2,850 Cal.	61.95	12.02	1,955	10.96		
	62.00		1,725	9.68		
			1,410	9.76		
			Total 11 days	135.62		
Basal + 2,850 Cal.	60.50	11.98	1,740	9.36		
	60.55		1,770	10.89		
	60.25		1,568	10.54		
	60.30		1,550	10.56		
	60.60		2,262	9.94		
	60.40		1,725	10.30		
			Mean	10.30	1.21	11.51
Basal + 1 litre raw milk 3,530 Cal.	60.50	17.79	1,677	11.73		
	60.70		1,628	12.92		
	60.60		1,731	12.95		
	60.75		1,445	13.17	1.560	
	60.90		1,844	13.36		
	61.15		2,012	14.04		
	60.55		1,728	13.47		
	61.15		1,610	13.00		
			Total 8 days	104.64	12.48	117.12
Basal 2,850 Cal.	61.25	11.98	1,940	10.89		
	61.15		1,840	10.27		
	61.15		1,548	10.38		
			Total 11 days	136.18		

ject 5 the urinary N output gradually rose till the 5th day when a more or less constant excretion was maintained. Subject 6 on the other hand exhibited a fall in N excretion about the 6th day. This fall occurred with both raw and boiled milks. On return to basal diet the excretion of N by Subject 6 dropped rapidly and two days after the cessation of the extra intake basal level was reached. Three days following the cessation of the extra milk N by Subject 5 basal level had not quite been reached, there still being a difference of about 0.3 g. N excreted in excess.

It is apparent that a very definite retention of milk N occurred during these experiments and that the retained N appeared to remain stored within the organism on return to basal diet. The evidence on this latter point was somewhat equivocal.

In computing the amount of retained N difficulty was experienced in deciding the most accurate method of analysing the data. We have expressed the data in three forms - a balance being struck in each instance:-

(1) Let total intake over period of 11 days (during first 8 of which extra milk ingested) = X_1, and total output during the same period (urine + faeces) = Y_1, then retention = $X_1 - Y_1$. If extra milk N ingested = Z_1, then percentage milk N retained = $\frac{(X_1 - Y_1) \times 100}{Z_1}$.

(2) Let total output during 11 days (urine + faeces) = X_2, and basal output during 11 days (urine + faeces) = Y_2, then excess output = $X_2 - Y_2$.

If extra milk N ingested = Z_2 then extra milk N retained = $Z_2 - (X_2 - Y_2)$ and percentage milk N retained = $\frac{Z_2 - (X_2 - Y_2) \times 100}{Z_2}$.

(3) On the evidence that the extra milk intake involved but little change in the faeces and since in any case no account had been taken of sweat N and cutaneous desquamation, it may be legitimate to consider the urinary excretions only.

Let total urinary excretion over period of 11 days = X_3, and basal urinary excretion over period of 11 days = Y_3, then excess output = $X_3 - Y_3$.

If extra milk N ingested = Z_3, then extra milk N retained = $Z_3 - (X_3 - Y_3)$ and percentage milk N retained = $\frac{Z_3 - (X_3 - Y_3) \times 100}{Z_3}$.

For comparison we have calculated the retentions by all three methods (Tables 15 and 16).

The main objection to the first method of computation is that no account is taken of the N in the sweat and cutaneous desquamation, so that the retentions observed appear slightly higher than they would actually be. The significance of the data derived by all three methods is naturally dependent on whether N retention was occurring under basal conditions. If a positive or negative retention did occur, then methods 2 and 3 would be least likely to give erroneous data regarding the amount of extra milk N retained. It was therefore necessary to note what was happening under the basal conditions (Table 14).

TABLE 15.

Subjects 5 and 6.

N balance during 8 days' extra raw milk intake and during first 3 days of subsequent basal period according to the three methods of computation.

Method	Subject	Total intake g.	Output g.			Retention g.	Extra intake g.	% extra N retained
			Urine	Faeces	Total			
1	5	209.59	164.50	21.60	186.10	23.49	46.24	50.8
	6	178.46	135.62	16.36	151.98	26.48	46.24	57.3
		Basal excretion						
2	5	160.48	164.50	21.60	186.10	20.62	46.24	44.6
	6	121.66	135.62	16.36	151.98	15.92	46.24	34.4
		Basal urinary excretion	Total urinary excretion		Extra urinary excretion			
3	5	140.03	164.50		24.47	21.77	46.24	47.1
	6	108.35	136.62		27.27	18.97	46.24	41.0

TABLE 16.

Subjects 5 and 6.

N balance during 8 days' extra boiled milk intake and during first 3 days of subsequent basal period according to the three methods of computation.

Method	Subject	Total intake g.	Output g.			Retention g.	Extra intake g.	% Extra N retained.
			Urine	Faeces	Total			
1	5	211.15	164.69	26.36	191.05	20.10	46.48	43.2
	6	178.30	136.18	16.11	152.29	26.01	46.48	55.9
		Basal excretion						
2	5	163.76	164.69	26.36	191.05	19.19	46.48	41.3
	6	126.61	136.18	16.11	152.29	20.80	46.48	44.8
		Basal urinary excretion	Total urinary excretion	Extra urinary excretion				
3	5	142.12	164.69	22.57		23.91	46.48	51.4
	6	113.30	136.18	22.88		23.60	46.48	50.8

TABLE 14.

Basal N Balance.

Subject	Period	Output g.			Intake g.	Retention g.
		Urine	Faeces	Total		
5	Pre-raw	12.73	1.97*	14.70	14.85	+0.15
	Pre-boiled	12.92	1.97	14.89	14.97	+0.09
	Pre-meat (1)	12.26	1.51	13.77	14.97	+1.20
	Pre-meat (2)	12.46	1.765	14.225	15.23	+1.005
6	Pre-raw	9.85	1.21*	11.06	12.02	+0.96
	Pre-boiled	10.30	1.21	11.51	11.98	+0.47
	Pre-meat	9.67	1.18	10.85	12.11	+1.26
	Pre-soya	11.09	1.12	12.21	12.11	-0.10

* The basal faecal N was not determined during the pre-raw period. The value obtained during the pre-boiled period is adopted.

The apparent retention of +0.15 g. and +0.09 g. N per day noted in the pre-raw and pre-boiled milk periods of Subject 5 point to slight negative balances, since the cutaneous N losses must have exceeded these values. In the case of Subject 6 true balances probably existed, for the second milk period was carried through during a colder spell of weather than the first and hence there would be less loss of N by the sweat. The daily excretion of N in the sweat varies with the activity of the subject and with the atmospheric conditions. Cuthbertson and Guthrie (1934) have also shown that the level of protein intake has apparently a direct influence on the N loss. In a series of normal men confined to bed, the N loss in the sweat (no account being taken of the nature of the diet) varied from 0.215 to 0.458 g. per day. The experimental conditions were somewhat

abnormal but the data obtained probably represent as true values as can be obtained for a 24-hours' continuous collection. Bost and Borgstrom (1926) found that two subjects doing office work and walking to and from their work in the morning and evening, lost during 24 hours 0.900 and 0.613 g. N in their sweat, the average air temperature being 29.4° and the relative humidity 67°.

The work done by Subjects 5 and 6 was harder than would be involved in office work, but on the other hand the air temperature was much lower. Taking the sweat N loss as 0.5-1.0 g. per day it would appear that in Subject 6 N intake practically equalled N output on basal diet.

If the mean per cent. retention for each subject be calculated, using the values derived by the three methods of computation, it is found that 47.5 and 45.3 per cent. of the extra raw and boiled milk N were respectively retained by Subject 5 and by Subject 6, 42.2 and 50.3 per cent. These retentions are of approximately the same order and confirm the marked storage of superimposed milk N witnessed in the earlier series, and substantiate the fact that no real difference exists between the nutritive values of the proteins of the two milks, as determined by this method in the adult or adolescent subject.

These extraordinary N retentions were coupled with increases in body weight. Utilising Rubner's value of 1 g. N as being equivalent to 33 g. body substance we have calculated the expected weight increase from the N retentions as determined by Method 2 and compared it with the actual (Table 17).

TABLE 17.

Body weight changes.
kg.

Raw milk.					Boiled milk.			
Subject	Basal weight	Weight after extra raw	Weight increase	Calculated weight increase	Basal weight	Weight after extra boiled	Weight increase	Calculated weight increase
5	86.40	87.12	0.720	0.680	86.45	87.0	0.550	0.640
6	61.45	61.85	0.400	0.530	60.45	61.20	0.700	0.690
Meat expt. over equivalent period.								
6	61.58	62.35	0.770	0.460				

It is apparent that the calculated data fit the actual with a reasonable degree of accuracy considering the many factors which may influence body weight. The increments in weight of Subject 5 were not maintained on return to basal, there being an immediate loss of weight. No significant difference could be detected in the alterations in body weight which occurred through feeding raw and boiled milks in this case. The fact that the rise in body weight was not maintained on return to basal suggests that the bulk of the material stored during the period of surfeit feeding was apparently not required by the organism to improve its 'nutritive state'. The fact that the stored N was apparently held fast as judged by the rapid return of the urinary N to practically its basal value, suggests that the N may be

stored in the form of "Meliorationseiweiss". On the other hand, there may have been a slow elimination of this stored N, for the period was not continued sufficiently long to rule this out of account.

The assumption has been made in all these experiments that the additional milk protein is all absorbed. In view of the fact that there was a slight increment in the faecal N during the periods of extra milk intake, this assumption may perhaps appear unjustified. It is uncertain, however, how much of this additional faecal N may have arisen during the passage of the increased bulk of mineral matter down the alimentary canal. Even if this additional faecal N represents unabsorbed milk N, the loss by this route would not have exceeded 8 per cent. of the additional N intake.

In the case of Subject 6, the weight changes were rather peculiar in that the steady gain in weight on both types of milk was broken on the seventh day of additional milk. This loss in weight was transitory, being made good the day following. On return to basal conditions the body weight did not fall but was maintained during the two to three days of the post-milk period. This is in striking contrast to the marked fall in body weight of Subject 5 on the first day of the post-milk period. This fall was not coupled with a diuresis and so it is not accounted for by a loss of water.

In a subsequent experiment Subject 5 consumed daily, beef + lactose + butter, equivalent in protein content and calorie value to a litre of milk. Again there was no fall in body weight

during the period of observation following the resumption of the basal dietary. The presumption is that we are dealing here with the reaction of the adolescent in contrast to the adult as 5 was aged 36 years and 6, 20 years.

This divergence in behaviour of the two subjects is of considerable importance, for in both subjects and with both types of milk the urinary N excretion returned approximately to basal following the withdrawal of the extra milk. At least this is fairly certain in the case of Subject 6, but with Subject 5 as previously mentioned there is some slight doubt, for on the third day of the post-milk period the urinary N level was still some 0.3 g. above the pre-period level. It would appear that part of the N gained during the period of extra milk feeding might continue to be retained for a time, even although the body weight might return to practically its basal value. This suggests that either (1) the retention of the N is not related intimately in space and time with the general gain in organic matter, or (2) that the N is preferentially stored when the extra food supply is withdrawn. Further discussion on this interesting point is meantime deferred.

The metabolism of calcium following the superimposition of raw and boiled milks on a diet adequate for maintenance.

In view of the marked retentions of milk N/it was considered of interest to determine if the same phenomenon occurred with the Ca of the milk. For this purpose the Ca balance was investigated during the periods of high milk intake.

The data for Subject 5 are given in the following table:-

TABLE 18.

Ca g.		
Raw milk.	Boiled milk.	
1.596	1.323	Urine (8 days)
17.254	17.520	Faeces (8 days)
18.850	18.843	Total excretion
9.666	9.495	Basal excretion
9.184	9.348	Extra Ca excreted
9.797	9.912	Extra Ca intake
0.613	0.564	Milk Ca retained
6.26	5.69	% retained

It is obvious that compared with the N retentions the retentions of Ca are slight, and indicate no real difference between raw and boiled milks.

Review of previous investigations to determine if the nutritive value of the proteins of milk is lowered by boiling.

As the composition of the milk of any species of mammal is peculiar to that species, being intimately related to the growth of its young, it is obvious that the ideal subject for an experiment designed to determine the nutritive value of raw and boiled milk is the weaned suckling of the same species. If the milk is fed to the young of a different species the composition of the milk should be considered so as to ensure that, if possible, some degree of similarity exists between the milk of the species itself and that of the milk to be used for feeding. This is from the theoretical standpoint. In actual practice it is somewhat surprising how well children grow on cow's milk, a milk whose composition is markedly different from that of woman's. Attempts to 'humanise' cow's milk are unsatisfactory and of doubtful value. Lane-Claypon from data which she collected in 1911 from a Berlin clinic, found that infants fed on boiled milk as compared with breast fed, had such favourable results as to render it unlikely that any form of artificial feeding would have produced as favourable results. "Where artificial feeding must be employed there is no evidence that milk loses any of its nutritive value by boiling. The work of numerous observers indicates that rather more satisfactory progress is

made with boiled than with raw."

Krost (1913) supervised the feeding of some 500 babies whose diet was controlled by him at an infant welfare station. Some of the infants got raw, others boiled. The gain in weight was greater in the boiled group.

Some animals are born comparatively mature, while others are very immature at birth. For example, guinea pigs are active almost at once after they are born, and are capable of eating almost anything. They receive mother's milk only for a short time and at no time are entirely dependent upon their mother. Many other laboratory animals on the other hand, require care and sustenance from their mother for a time.

As Lane-Claypon (1916) has pointed out, "It is to some extent irrelevant to compare for instance, the nutritive value of raw and boiled cow's milk upon guinea pigs or rats, with a view to ascertaining the value of this food for infants. Rats and mice reach maturity in a very few weeks from the time of their birth. An age difference between different batches of such laboratory animals of even a day or two might destroy the value of the experiment. Too much stress cannot be

laid then on such experiments. Indeed, very few experiments have actually been designed to determine if the quality of the protein of milk is altered by boiling; most experiments have been concerned with the general nutrition of the subjects investigated.

It will be most convenient to consider briefly (1) the nature of the changes, if any, produced in the protein moiety of the milk by boiling, and (2) the effect of boiling on the retention of the milk N.

(1) Boiling milk causes one certain change in the protein fraction of milk as will be observed from this modified table of Kieferle and Gloetzl (1930), viz. coagulation of a considerable fraction of the lactalbumin (and also lactglobulin).

Constituent	Raw	Boiled
Total N (mg. N %)	540.4	540.5
Casein	348.3	383.0
Albumin	75.7	13.9
Albumose	44.6	42.4
Total residual N	123.6	152.0
Amino	4.12	5.45

The only significant change then is the diminution in the albumin fraction which decreased from 75.7 to 13.9 mg. N per cent., a fact confirmed by many other workers, and observed qualitatively by every housewife. It is of interest to mention here, that a small quantity of calcium and phosphorus is rendered

insoluble on boiling milk. This was first noted by Soldner (1888) who thought it might be due to precipitation as tri-calcium phosphate. Subsequent workers have confirmed this fact but no analytical evidence has yet been brought forward to prove that the precipitated substance is tri-calcium phosphate.

(2) Aron and Frese (1908) found in two female pups fed on diets in which the only source of Ca was raw milk, sterilized milk or solid tertiary calcium phosphate with dried flesh bringing up the caloric values of the diets to the same level, that the weight increase and N retention favoured the calcium phosphate period and was least in the raw period!

The writer is in agreement with Stirling and Blackwood (1933) that no conclusions can be based on Cronheim and Müller's (1908) experiments on the effect of raw, boiled and sterilised milks on the mineral, fat and protein metabolism of normal and rachitic children. In Daniels and Stearn's (1924) investigation on children, no general statement could be made about the effect of boiled milk on N retention, but as regards Ca and P they had no doubt but that under the conditions of their experiment, boiled milk enabled the infants to store more Ca and P than did milk pasteurised by the "holding" method. The next year Terroine and Spindler (1925) noted that heating milk to 95°C. for 1-2 minutes did not affect the absorbability of the proteins from the intestines of three growing pigs. Morris and Graham (1933) have recorded metabolism experiments on two

apparently healthy babies, aged 7 and 8 months. These were fed on an adequate measured amount of boiled cow's milk for 10-14 days, on the last seven of which excreta were collected. Thereafter the same amounts of raw milk obtained from the same source were given for a fortnight, the excreta as before being collected on the last seven days. Their results indicate that there is no evidence to support the idea that boiling milk interferes with its usefulness as a food for infants. In the following table are given their data:-

Subject	Diet	Retention per kg. per day.				Gain in wt.
		N	CaO	P_2O_5	Fat	
1		g.	g.	g.	g.	g.
Period 1	Boiled	0.162	0.060	0.0575	3.8	150
Period 2	Unboiled	0.071	0.041	0.0410	3.85	140
2						
Period 1	Boiled	0.194	0.0003	-0.0098	3.75	100
Period 2	Unboiled	0.125	-0.009	-0.090	3.70	250

Morris and Graham (1936) have recently repeated these experiments with substantially the same result.

According to the work of Symington (1936) the rate of digestion of the proteins of milk in the stomach is unaltered by boiling, and Munro (1936) has detected no significant difference in the specific dynamic actions exerted by the raw and boiled milks following the absorption of the products of their digestion.

It would be out of place here to enter into a detailed analysis of the investigations planned to determine if the boiling of milk alters its general nutritive value, for this involves the consideration of many other factors, notably the vitamin C content. If the vitamin C deficiency is made good, the general consensus of opinion is that boiling does not interfere with the usefulness of milk for infant feeding.

We may conclude this chapter with the observations of the Advisory Committee on Nutrition as incorporated in their First Report (1937):

> "The effects of heat on the nutritive value of milk for animals have been extensively studied, but the few human experiments that have been done have not shown that heat significantly lowers the food value of milk for man. This does not necessarily mean that heated milk is equal in all respects to raw milk, since such a conclusion could only be drawn if we knew all there is to be known about human nutrition and about the nutritive properties of milk, and this is far from being the case. So far as is known, the only significant changes effected in the composition of milk are a partial loss of vitamin C and possibly iodine. The amount of vitamin C in raw milk is in any case small and even in raw milk some loss usually occurs in course of distribution. ..... It is therefore reasonable to assume that heated or dried milk or milk incorporated in other cooked articles of diet

such as bread and puddings retains most of the nutritional properties of raw milk." *

There is therefore no reason to wonder that the experiments on raw and boiled milk, which have been described in the earlier pages, yielded no evidence of a change in the nutritional value of the proteins caused by boiling. It would have been an extraordinary thing if they had, for the choice of subject was unsuitable, an adult instead of a child, and the time of observation was too short. The remarkable fact is, however, the unexpected and considerable retention of the extra nitrogen and sulphur, a retention unaffected by boiling the milk. Is this storage phenomenon peculiar to milk when superimposed on an adequate diet or is it merely an example of a more general reaction? This was the question which demanded further enquiry. In the following pages are described the experiments which were designed to elucidate this point.

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* The recent work of Kon and Watson (Biochem. J. 1936, 30, 2273) has shown that a pint bottle of milk exposed under practical conditions on the doorstep for half-an-hour in the sun and then kept in the darkness for 1 hour loses fully half of its original antiscorbutic properties.

PART 2.

I. THE SUPERIMPOSITION ON A DIET ADEQUATE FOR MAINTENANCE OF BEEF + LACTOSE + BUTTER EQUIVALENT IN PROTEIN, CARBOHYDRATE AND FAT CONTENT TO A LITRE OF MILK.

EXPERIMENTAL.

In the first part of this work attention has been directed to the remarkable retention of N and S which may occur in the adult human organism during the superimposition of milk on an already adequate diet. The question naturally arose whether this marked storage of N would occur when some other protein of animal origin was superimposed. To determine this point the experiments were repeated, superimposing on the same basal diet lean beef with supplements of lactose and butter, so that the final quantities of protein, fat and carbohydrate ingested daily were equivalent to these principles in the extra litre of milk. For this, 160 g. lean topside beef (av. T.N. = 5.68 g.), 46 g. lactose and 35 g. butter were required. Water equal in volume to the water content of the milk was drunk at times corresponding to the extra ingestion of milk.

Owing to an attack of influenza Subject 5 had to abandon his experiment after 3 days of extra meat, butter and lactose had been ingested.

TABLE 19.

Subject 5.

Superimposition of beef + lactose + butter over period of three days.

Diet.	Daily N intake g.	Daily Urinary N g.
Basal	14.97	12.28 12.24
		Mean 12.26
Basal	20.82	13.66
160 g. beef (lean topside) + 46 g. lactose + 35 g. fresh butter		14.29 14.29

During the short period of observation N retention very definitely occurred. The time allowed for the establishment of equilibrium would probably have exceeded the three experimental days.

Over a superimposition period of 8 days Subject 6 exhibited a retention of N representing 31.4 per cent. of the additional intake. On a similar basis the retentions observed with raw and boiled milks were for this subject, 36.3 and 46.1 per cent. respectively (Table 20) that is when computed by Method 2.

TABLE 20.

Subject 6.

Comparison of N balance for first 8 days of extra raw and boiled milks and extra beef + lactose + butter.

Period	Output			Basal output for corresponding period g.	Extra N intake (8 days) g.	Retention g.	% extra N retained
	Urine g.	Faeces g.	Total g.				
8 days extra raw milk	105.22	12.73	117.95	88.48	46.24	16.77	36.3
8 days extra boiled milk	104.64	12.48	117.12	92.08	46.48	21.44	46.1
8 days + extra beef, lactose, and butter	108.47	10.40	118.87	86.88	46.60	14.61	31.4

In order to determine the effect of this continued superimposition, the additional food was continued for a total period of 15 days (Table 21, Fig. 11).

TABLE 21.

Subject 6.

Superimposition of beef + lactose + butter.

Day of Expt.	Daily diet.	Body weight kg.	Daily N intake g.	N output daily g.			
				Urine vol.ml.	Urine	Faeces	Total
1	Basal	62.05		1520	10.06		
2	(2850 Cal.)	62.20		1660	9.53		
3		61.70	12.10	2190	10.30		
4		61.30		1930	9.15		
5		61.65		1240	9.39		
6		61.65		1571	9.60		
7		61.45		1565	9.63		
8		61.25		1589	9.77		
Average (1-8 days)		-			9.68	1.18	10.86
9 -	Basal + 160 g.	61.20		1794	12.93		
10	beef + 46 g.	61.20		2052	13.10		
11	lactose + 35 g.	61.40		1583	13.34		
12	butter (3550 Cal)	61.60	17.925	1570	13.57		
13		61.90		1620	13.95		
14 -	Lactose replaced	62.25		1577	13.69		
15	with butter	62.35		1835	13.77		
16		62.20		1847	14.12		
Total (9-16 days)		-			108.47	10.40	118.87
17	Lactose restored	62.45		1630	13.16		
18		62.45		1790	14.38		
19		62.50	17.925	1907	13.54		
20		62.60		1490	14.42		
21		62.60		1725	14.23		
22		62.50		1425	14.25		
23		62.50		1895	14.84		
Total (9-23 days)		-			207.29	19.50	226.79
24	Basal	62.30		2052	11.00		
25	(2850 Cal.)	62.40		1757	10.48		
26		62.45		1847	10.49		
27		62.75	12.10	1680	11.03		
28		-		1720	10.63		
29		62.45		1740	10.54		
30		62.30		1907	10.30		
31		61.65		1925	10.81		
32		61.35		2195	10.41		

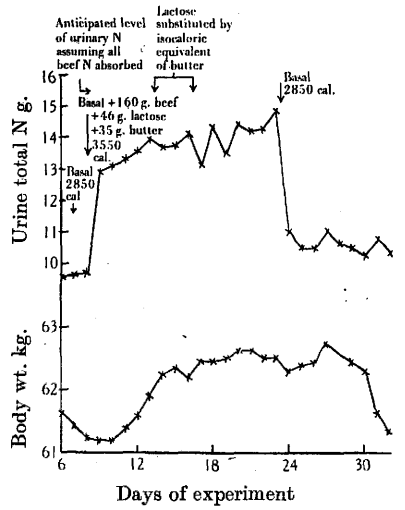


Fig. 4. The effect of superimposing beef + lactose + butter daily equivalent in protein, carbohydrate and fat to a litre of milk. Subject 6.

TABLE 22.

Subject 6.

N balance during 15 days' extra beef intake and during first 3 days of subsequent basal period according to 3 methods of computation.

Method	Total intake g.	Output g.			Retention g.	Extra intake g	% extra N retained	Body wt. increase
		Urine g.	Faeces g.	Total g				
1	305.17	239.26	23.04	262.30	42.87 (1430 g. flesh*)	85.37	50.2	circa 1,000 g.
2	Basal excretion 195.48	239.26	23.04	262.30	18.55 (618 g. flesh*)	85.37	21.7	
3	Basal urinary excretion 174.24	Total urinary excretion 239.26	Extra urinary excretion 65.02		20.35 (680 g. flesh*)	85.37	23.8	

* On basis of 1 kg. body substance = 30 g. N.

The urinary N excretion slowly rose reaching a maximum value on the fifteenth day of the experiment - a value still short of that to be expected if N equivalent in amount to the daily additional intake of N were excreted. N equilibrium was therefore still unattained although the degree of retention was steadily becoming less. Equilibrium would eventually have been achieved. On reverting to the basal intake again the curve of urinary N fell to a new basal level in the course of two days.

A slow relatively steady elimination of the retained N appeared to take place, so slow that it scarcely affected the body weight, for coupled with the retention of N there had occurred an increase in body weight which was maintained during the subsequent first 8 days of the basal period. The body weight fell on the 9th day of basal diet and by the following day had reached its original level. In this respect the experiment corresponds to the previous experiments on Subject 6. The actual body weight increase was about 1 kg. whereas that calculated from the N retention as found by Method 2 gave a value of 618 g., that is on the assumption that the material stored has a N content comparable to muscle; by Method 3, 680 g. In a prolonged experiment of this nature it is not to be expected that the material stored at the beginning of the experiment would have the same composition or storage site as that retained during the later stages.

Exchanging an isocaloric equivalent of butter for the lactose did not appear to affect the trend of the N retention, apart from causing an initial lag in N excretion.

The data have been subjected to the same three methods of computation, Table 22. It will be noted that by Method 1, i.e. based on differences between total intake and total output, the percentage retention of the extra N is 50.2, whereas by the other two methods much lower values are obtained, due to the fact that no account has been taken in Method 1 of the cutaneous N loss, which over a period of 15 days would be quite considerable. This discrepancy emphasises the unsuitability of basing retention values on the difference between total intake and total output, as represented by the sum of urine N + faecal N. Method 2

appears then to offer the best method of determining retention values if data for faeces are available. By this method the retention of N in this experiment was 21.7 per cent. over the period of 15 days, account being taken of the first three days of return to basal diet. The retention during the first 8 days was 31.4 per cent. of the additional intake.

In the light of our observations we have analysed the data accumulated by Wilson (1930,1931) in the course of his study of the physiology of protein retention. Wilson's basal diet consisted of 470 g. bread, 125 g. butter, 200 g. jam, 1 apple (total Cal. 2500). His initial body weight was 65 Kg. The basal diet therefore had 38.5 Cal./Kg. When equilibrium was reached on the basal diet a supplement of 250 g. beef was superimposed daily for 4 days. Thereafter another 250 g. beef was added daily for 5 days. These supplements were then removed stepwise. It is evident from his first experiment that a very marked retention of N and S took place even when a diet rich in first class protein (500 g. beef ≡ 108 g. protein) replaced a diet of average first class protein content (250 g. beef ≡ 54 g. protein).

Wilson's second experiment on himself which is more comparable to ours, again revealed an extraordinary retention of N and S, a retention which still obtained on the eighth day of supplementing the basal ration with 250 g. beef per day. From the course of the gradually rising daily urinary N excretion it would appear that given sufficient time, N and S equilibrium

would have been reached. The somewhat similar experiment on Subject 6 in our series, indicated that even after 15 days of extra beef ingestion N equilibrium might still be unattained. In this latter experiment in addition to the beef supplement, fat and carbohydrate of equicaloric value to the fat and carbohydrate of a litre of milk were consumed. The extra calories probably account for the difference in the rates of N excretion in these two experiments. The present writer does not consider the method which Wilson ultimately used (1931) to calculate his N and S retentions as the better of the two methods he originally described (1930). The method adopted obscured the extent of the retention. Wilson assumed in his first experiment that the daily urinary excretion of 12.235 g. N represented the point of N equilibrium following the consumption of 250 g. beef. Taking his basal value of 6.206 g. the extra N eliminated in the urine at this point is 6.029. The extra N intake was 8.620 g. This difference between intake and output is not however mentioned specifically by Wilson (1931). He assumed that when equilibrium was attained the N and S outputs in the urine represented the total N and S absorbed from the alimentary canal. Although Wilson did not determine the N content of his faeces it is highly improbable that an increase in faecal N of the extent required to balance intake and output would result from defective absorption. In his case it would mean a coefficient of absorption of only 75%. Wilson evidently did not consider this too low a value for beef. We have recalculated Wilson's second experiment (Table 23)

Data derived from Wilson's theorie (1950).

Legend Mixing M (g cm^{-3}) Solid particle density Liquid M (g cm^{-3}) Solid particle density	Volume fraction solid particles in feed Volume fraction liquid in feed	Liquid M (cP) Solid particle diameter Liquid M (cP) Solid particle diameter	Settling velocity Settling velocity
1.38 2.65 1.00 2.65	0.500 0.494	1.00 0.04 1.00 0.04	4.40 1.85e

TABLE 23.

Data derived from Wilson's thesis (1930).

Basal urinary N (8 days).	Total urinary N during period of extra beef.	Extra N output.	Extra N intake.	Retention of N.	Percentage retention	S:N ratio of material retained.
52.45	81.00	28.55	68.96	40.41	58.6	1:23.98
Basal urinary S (8 days).	Total urinary S during period of extra beef.	Extra S output.	Extra S intake.	Retention of S.	Percentage retention	
4.016	6.560	2.544	4.400	1.856	42.2	

on the basis that all the additional N and S ingested was absorbed. From our experience of the relative constancy of the faecal N it is unlikely that this assumption can be far wrong. The N and S retentions as determined by this method are marked and comparable to those we have just described. Wilson's data indicate, when recalculated, an S:N ratio of 1:23.98 for the material stored during the period of 8 days - a value much lower than that found by the method he adopted, namely 1:16.76. Wilson's experiment therefore lends support to our observations and is of particular interest in indicating that with the lapse of time the retention diminishes.

PART 2.

II. THE EFFECT OF THE SUPERIMPOSITION OF SOYA FLOUR + LACTOSE + BUTTER ON A DIET ADEQUATE FOR MAINTENANCE.

Recently much attention has been directed to the cheapness and nutritional value of the soya bean which has made it indispensable in the diet of many eastern races. Its amino acid composition is closer to that of beef than either wheat or rice. The chief protein, glycinin, contains from 1.89-2.84 per cent. tryptophane, 0.74-1.46 per cent. cystine and 3.94-4.55 per cent. tyrosine according to the relative proportions in which the different globulins comprising the glycinin fraction are present in the different varieties of the bean (Csonka and Jones, 1933). It was thought of interest to repeat our experiments using soya flour. The flour selected was a patented variety "Soyolk" containing 42.12 per cent. protein, 19.64 per cent. fat, 1.38 per cent. lecithin, 23.40 per cent. N free extractives (made up of 4.5 per cent. total sugars, 3.1 per cent. dextrin, 4.9 per cent. pentosan, 4.9 per cent. galactan, 3.3 per cent. cellulose, and 4.76 per cent. ash). This flour is practically free from the specific taste of the soya bean.

As early as 1912 Osborne and Mendel pointed out that at least one of the proteins of the soya bean - glycinin - could facilitate growth satisfactorily but as the sole article of a dietary it was deficient in mineral constituents, being relatively poor in Ca and Cl. On diets containing the soya bean meal together with fats and "protein-free milk" or an artificial salt mixture, several broods of vigorous young rats have been produced and these have grown normally on diets the same as

those on which their parents were raised. This is in striking contrast to the adverse effects of kidney beans and garden beans (Osborne and Mendel, 1917). Johns and Fink (1921) have also shown that for rats bread, with a mixture of 25 parts soya bean flour and 75 parts wheat flour, was adequate for normal growth. In 1932 Mitchell and Smuts (1932) adduced evidence from their rats' experiments which indicated that both beef and soya protein were deficient in cystine, the soya more so than the beef. The experiments of Tomhave and Mumford (1933) have thrown doubt on the complete nutritional excellence of this bean in five large scale experiments on growing chicks. Protein supplements of meat scrap and dried buttermilk were compared with replacements of these, in part or in whole, by soya beans. They found that soya beans could not be substituted for any of the buttermilk or for more than one third of the meat scrap without seriously affecting growth, mortality or utilisation of the feed. Raw soya beans have been found unsatisfactory for growth and well-being of rats (Vestal and Shrewsbury, 1932).

From experiments designed to determine the effect of the efficiency of utilisation of the N of different foodstuffs on the growth of young pigs, Terroine and Valla (1933) found that taking the utilisation of skim milk as 100, soya bean flour gave a value of 66.5, the highest for cereals and only slightly lower than that for egg white, viz. 70.8. Schmidt (1933) was of the opinion that for pigs soya bean meal could not wholly replace animal protein, but if milk was not available it could replace

half the usual quantity of animal protein.

In human nutrition there are a considerable number of observations to indicate that soya bean flour is of considerable biological value. Rose and MacLeod (1925) in experiments on three women subjects, have studied the N metabolism on diets, adequate in energy, containing practically all of their N in the form of lean beef, milk, bread and milk or soya bean curd. Although the N intake per kg. was less than the average for adult maintenance on a mixed diet as laid down by Sherman, in all cases the average N balance in periods of 12-15 days' duration, was positive. With two subjects slightly more favourable balances were obtained on milk than on meat diets, while with the third subject no clear distinction was evident. With one subject the bread and milk diet, containing approximately equal amounts of N from each food, gave almost as favourable a N balance as the milk diet; in another subject the soya bean diet induced a small positive N balance as well as a very low level of N in the urine. London et al. (1932) state that the food value of the soya protein for the human subject is 88 per cent. of that of meat and fish, and the carbohydrate food value they place at 100 per cent.

Rittinger, Dembo and Towey (1935) have found good weight curves in infants fed soya bean milk (grain pulverized in water and filtered) either alone or in combination with various percentages of skimmed milk.

EXPERIMENTAL.

Subject 6 took part in this experiment. The basal dietary was similar to that adopted in the previous experiments. In place of beef, 81 g. "Soyolk" (T.N. = 5.7 g.) were daily made into a soup, some onion and carrot being added to flavour and annul the beany taste. The vegetables were removed before consumption and 17 g. butter and 27 g. lactose added to bring the energy value up to that of a litre of milk. As difficulty was experienced in consuming this preparation owing to digestive disturbances, the period of superimposition had to be closed after five days. As in other experiments on Subject 6 this soya preparation was all consumed at breakfast. The water intake was kept at a constant level.

The pre-soya period was a continuation of the post-meat period of the previous experiment and was arbitrarily reckoned to commence 14 days after the conclusion of the extra beef intake. The full quota of "Soyolk" was not ingested on the first day of superimposition, the subject vomiting a small amount. This experiment is not therefore very satisfactory.

When the N balance for the 5 days of added soya flour and the first two days of the subsequent basal period is analysed by the three different methods of computation, it is found that the approximate percentage of the extra protein is 54.5, 33.7 and 41.9 by the respective methods (Table 24). The discrepancy between the first method of computation and the other two is again due to the fact that no account is taken of the cutaneous

TABLE 24.

N balance during 5 days of added soya flour and during first 2 days of subsequent basal period according to 3 methods of computation.

Method	Total intake	Urine	Faeces	Total	Extra intake	Retention	% extra N retained	Body wt. increase
1	113.45	90.65	10.14	100.79	27.85	15.19 (506 g. flesh)	54.5	
2	Basal excretion 82.32	90.65	10.14	100.79	27.85	9.38 (341 g. flesh)	33.7	circa 350 g.
3	Basal urinary excretion 74.48	Total urinary excretion 90.65	Extra urinary excretion 16.17		27.85	11.68 (389 g. flesh)	41.9	

Weight before additional protein = 61.90 kg.
" at close of " " = 62.25 kg.
" 3 days after " " = 62.20 kg.

loss in Method 1, whereas by the other two methods it is assumed to be constant throughout the experiment, and so does not affect the calculation. The discrepancy between Methods 2 and 3 is due to the increased faecal N excretion over basal which occurred during the period of soya feeding. As no account of this is taken in the third method of calculation the apparent retention is greater than the actual.

The increase in weight corresponds fairly closely with that calculated from the N retention, on the basis that the material retained is mainly muscle.

In Table 25 are recorded the retentions of the additional N as observed over 5 days' supplementary feeding of Subject 6 with raw and boiled milk, with meat and with 'Soyolk'. For comparison the retentions noted during similar periods of supplementary feeding of milk by Subject 5 are included. It will be noted that over this period slightly more N was stored by Subject 5 when on extra raw milk than when on extra boiled milk N, that is 59.6 against 52.6 per cent. The reverse held with Subject 6, the difference in this case, 34.6 against 50.5 per cent., being more marked than that found when calculated over eight days. During the 'Soyolk' period N was retained just as effectively as during the boiled milk period.

TABLE 25.

Comparison between retentions of N observed with milk, beef and 'Soyolk' over periods of 5 days as calculated by Method 2.

Subject	Source of additional protein	Extra Nitrogen g.	Retained N g.	Per cent. retention of extra N.
5	Raw milk	28.90	17.02	59.6
	Boiled milk	29.05	15.28	52.6
6	Raw milk	28.90	10.00	34.6
	Boiled milk	29.05	14.67	50.5
	Beef	29.13	10.04	34.5
	'Soyolk'	27.85	14.30	51.3

DISCUSSION.

These experiments have demonstrated that in the adolescent and even adult human subject, the superimposition of a litre of milk for one day on a basal diet, adequate for the maintenance of weight and N equilibrium and already containing 500 ml. raw milk, brought about retentions of N and S equivalent to 54 and 53% of the N and S added to the diet. No statistical difference could be detected between the retentions observed with raw and boiled milks. It is of interest that superimposed sodium caseinate, equivalent in N content to a litre of milk, was only half as well retained, indicating that probably the other constituents in the milk were playing a rôle in effecting this retention.

The maximum excretion of total N, total sulphates and urea occurred on the day on which the additional milk was ingested. On the succeeding day the level of total N and urea in the urine was still above normal but the sulphate excretion had declined to the basal level. The ammonia excretion was practically unchanged by such milk supplements - a fact which was rather to be expected. The reason for analysing the urine for ammonia was because some unpublished observations by another worker in this field had indicated that a disturbance of ammonia excretion might result from the addition of boiled milk. Our observations indicate that there are no grounds for such a belief.

Considerable variability in the response to even the same type of milk may be found. Subject 7 for example excreted

1.84 g., 2.21 g. and 1.25 g. N over the basal output on three separate occasions in response to a litre of raw milk. The analytical data of Subject 8 demonstrate how extremely variable may even be the basal excretion of total N. The occurrence of such wide fluctuations completely negatived further experimentation on this subject. This individual was a very reliable subject whose day to day energy expenditure remained very constant. Occasional records of experiences similar to this are found in the literature. They are quite inexplicable.

This unexpected retention of N brought about by the ingestion of additional milk was not followed by a period of increased elimination, at least so far as these observations go. In the light of subsequent experiments, it would have been advisable to have prolonged the post-milk period in order to determine if the excess N was slowly excreted over a long period of time. This N retention persisted even when the supplementing of the diet with extra milk was continued for eight days and exhibited no falling off in intensity. As was to be expected, again no real difference between the retentions of raw and boiled milk was detected. The percentage retention values for raw and boiled milks exhibited by Subject 5 were 44.6 and 41.3 respectively when calculated by Method 2 (page 44) which is the most satisfactory of the three described. This difference is not significant. With Subject 6 the discrepancy was rather wider. The percentage retention values for the latter were 34.4 and 44.8% respectively. If the values be calculated by Method 1 the differences in the values are exactly reversed. This is

apparent from Tables 15 and 16. It is fairly obvious that if such differences in these values be held to be significant, then the conclusion derived from assessing the data by Method 1 is exactly opposite to that derived from Method 2. We believe therefore that no real difference in the retentions of N effected by raw and boiled milks can be detected in such relatively short period experiments.

In calculating the retention of the additional N it has been assumed that all the extra N was absorbed. From the faecal analyses it is unlikely that on any day the additional milk N escaping absorption would have exceeded 8%.

The retention of calcium in contrast to that of N was relatively insignificant. 6.26% and 5.69% of the additional raw and boiled milk Ca were retained. These values are so low as to be practically within the bounds of experimental error. Examination of Table 18 reveals that with boiled milk rather more calcium was excreted by the bowel and rather less in the urine than was the case with raw milk. A repetition of this particular observation would be necessary before any real weight could be attached to this rather interesting finding.

An increment in body weight accompanied these extraordinary N retentions and it is of interest that the calculated weight increase, which is based on the assumption that 1 g. N is approximately equivalent to 33 g. body substance, mainly muscle, agrees fairly closely with the observed weight increase. This observation would suggest that the main weight increase in dry matter is not fat.

The weight increments brought about by the superimposed milks were not sustained on return to basal dietary conditions in the case of Subject 5. This was rather strange, for there was no obvious diuresis to account for it and no excessive elimination of nitrogenous material. The body weight record in this subject is not so complete as with Subject 6. Teaching duties frequently interfered with body weight determinations at the selected zero hour.

Subject 6 did not show any evidence of a return to basal weight level following the resumption of the basal diet, at least during the first three days of the post milk period. In the meat + lactose + butter experiment which followed a similar lag period was observed.

As has already been pointed out, these observations have revealed no real difference between the metabolic fate of the proteins of raw and boiled milks. This was to be expected, particularly as precautions were taken to consume the coagulated protein which stuck to the cooking vessel. This is a precautionary measure which unfortunately is not always observed in experiments designed to assess the nutritive values of raw and boiled milks. Frequently too, it is presumed that a given volume of boiled milk is identical to the same volume of milk boiled. For example, the loss of water during the process of raising a litre of milk fairly rapidly to the boil in an open aluminium pan, $8\frac{1}{2}$" in diameter, by the use of two Bunsen burners may amount to 50 ml. This represents a 5% concentration of the constituents. This degree of concentration is not proportional to the total

volume of milk, but amongst other things to the surface exposed. The degree of concentration with smaller volumes of milk will therefore be greater than 5%.

Comparison between the retentions of N brought about by additional raw and boiled milk with that observed on the superimposition of beef + lactose + butter over 8 days, indicated that the greatest retention was effected with boiled milk (46.1%). Raw milk occupied an intermediate position (36.3%); meat appeared to be least effective (31.4%). The writer believes that too much emphasis must not be attached to these apparent differences in the metabolic fate of these types of extra food N. Biological variation and experimental error may account for the observed differences.

Instead of bringing the beef experiment to a close after 8 days superimposition, the additional beef was continued for a further period of 7 days in order to determine whether N equilibrium would eventually be reached. Although the urinary N rose fairly steadily, N equilibrium was still not quite attained on the 15th day of extra beef + lactose + butter. This marked lag was unexpected, for it was not a case of supplementing a N-poor ration by a N-rich ration. The protein content of the basal diet was adequate both qualitatively and quantitatively. Wilson's data (1930, 1931) indicate that quite considerable though steadily diminishing retentions may be found even when beef is the sole supplement. During this period of surfeit the body weight of Subject 6 steadily rose, the actual weight increase being about 1 kg. The calculated weight increase based on the

N retention was 618 (Method 2). This discrepancy is greater than that noted in the milk experiments and probably denotes that an extensive deposition of fat or water or both was also taking place. On return to basal dietary conditions there was a slow, but relatively steady, elimination of the retained N, less than a gramme per day. There was no sudden drop in body weight. The gain in weight, just as in the milk experiments on this subject, was retained for some 5-6 days. A rapid fall then occurred without any definite change in the N elimination. This fall was largely due to a diuresis - a fact which indicates that during surplus food consumption a marked water retention occurs.

When discussing this particular experiment, it is illuminating to consider the very considerable divergence between the retention value obtained by Method 1, as compared with the other two methods of computation. It will be remembered that Method 1 is based on difference between intake and output, whereas the other two methods are concerned with the relationship between excess intake and excess output. Methods 1, 2 and 3 gave percentage retention values of 50.2, 21.7 and 23.8 respectively for the period of 15 days extra beef + lactose + butter and during the first three days of the subsequent basal period. As has already been pointed out, this considerable discrepancy is due to the fact that by Method 1 no account is taken of the cutaneous loss. In a long experimental period it may be quite considerable.

Soya flour + lactose + butter equivalent to the protein, carbohydrate and fat in a litre of milk also brought about a

marked retention of N when superimposed on an adequate basal diet. The calculated and actual body weight increments agreed very closely, and proved as effective as the milk and beef supplements in effecting a N-saving effect. As has already been mentioned, too much emphasis must not be laid on differences and similarities in retention values. Extraneous factors, particularly those which affect the faecal N excretion, may introduce variations in the day to day exchange. The main fact of importance is that here again evidence of a considerable N retention is forthcoming. It is impossible to define which protein moiety of these mixed diets was contributing mainly to the N retention. For example in this last experiment we are not justified in considering the N retained to be of 'Soyolk' origin. It may well have come from the protein of the milk in the basal dietary. The superimposition type of experiment must therefore be used with great caution for the determination of the nutritive value of a food stuff.

We may conclude by stating that when a dietary which is adequate for maintenance is supplemented by protein, whether of animal or vegetable origin, and particularly if the energy content of the diet is still further increased by additions of carbohydrate and fat, a considerable retention of N and S may result, the degree of retention bearing a relationship to the total increment in the energy value of the diet. In the case of beef + lactose + butter this retention of nitrogenous material diminished with the passage of time but was still considerable

even after fifteen days of surfeit. The nature and site of the retained material will be discussed at the conclusion of Part 3.

SUMMARY.

1. The superimposition of a litre of raw or boiled milk on a diet adequate for maintenance of body weight and N equilibrium in the adult human subject, caused an increase in body weight and a marked retention of N and S but not Ca. The addition of sodium caseinate equivalent in N content to a litre of milk to the diet of one subject, resulted in a definite but much less marked storage of N.

2. Comparison of the N retentions effected by the addition of milk, whether administered for one day or eight consecutive days, revealed no real difference in the metabolic fate of the proteins of raw and boiled milk.

3. The superimposition of beef + lactose + butter, equivalent in protein, carbohydrate and fat content to a litre of milk, also effected a definite saving of food N which was still in evidence even after fifteen days of surfeit. Soya flour + lactose + butter achieved a similar effect.

4. On resumption of basal conditions the retained N was apparently eliminated at a slow even rate.

5. The use of the superimposition type of experiment in nutritional studies is discussed.

PART 3.

THE PROTEIN SAVING EFFECT OF CARBOHYDRATE AND FAT WHEN SUPERIMPOSED ON A DIET ADEQUATE FOR MAINTENANCE.

Review of literature bearing on the protein-sparing effect of carbohydrate and fat.

In the foregoing pages of this thesis, attention has been drawn to the fact that retention of N by the adult organism readily occurs when excess protein-containing foodstuffs are added to an apparently adequate diet. The fact that one of the subjects retained 33 per cent of the N added to his basal diet in the form of sodium caseinate, in contrast to a retention of approximately 60 per cent observed with milk of equivalent N content, suggested that the non-protein fractions of the milk were playing a significant rôle, probably in virtue of their energy value. We have already cited the evidence in the literature which lends support to this belief, in particular the experiments of Voit, Rubner, Deiters, Neumann and Grafe. These experiments indicate that an individual, subsisting on a diet sufficient to maintain weight and N equilibrium and with complete metabolism of the proteins of the food, may retain a certain proportion of the food N if given a larger amount of N-free food material with or without additional protein. If the additional carbohydrate and/or fat be insufficient to cover more than the specific dynamic action of the additional protein, no storage can take place. It is apparently necessary to give large quantities of carbohydrates or fats to save small quantities of protein. According to Voit and Korkunoff (1895) not more than 15 per cent. of the previously metabolised protein can be spared by such additions. On the other hand, even up to 50 per cent. of the total N excreted during complete starvation may be spared by giving a free supply of carbohydrate.

In fasting experiments the amount of stored glycogen and fat in the organism exercises a "sparing" influence upon protein metabolism, the amount of protein catabolised being smaller the more abundant the store of glycogen and fat. Similarly the amount of carbohydrate and fat in the food conditions the N balance.

The protein-sparing effects of carbohydrate and fat have frequently been compared and the differences in the extent of their action have been shown in three main ways:-

(1) By adding or withdrawing an isodynamic quantity of fat or carbohydrate from a diet which has been given for some time, and which is just sufficient to maintain the organism in equilibrium.

As far as the present writer knows, Voit's experiments on the dog constitute the only evidence available on the relative effects of the addition of carbohydrate or fat to an adequate diet. The conditions were not exactly reproduced in the experimental series so that it is not possible to compare them accurately. The following table is taken from v. Noorden's "Metabolism and Practical Medicine" and represents an attempt at comparison.

Duration	Diet g.			Metabolism of meat.
	Meat	Fat	Starch	
4 days	1,500	-	-	1,774
5 "	1,800	250	-	$\frac{1,634}{140\ g}$ = 30g. protein saved
3 "	1,000	-	-	1,028
3 "	1,000	-	100-400	$\frac{902}{126\ g}$ = 27g. protein saved.

Lusk's (1890) experiments on himself, which were done at the suggestion of Voit, demonstrated the susceptibility of the protein metabolism to the sudden withdrawal of carbohydrate but no comparison was made with fat.

(2) By comparing the effects of giving equivalent quantities of carbohydrate and fat to the starving organism.

Rubner (1883) was the first to direct attention to the fact that the protein metabolism of the fasting dog could be reduced to 4 per cent. of the total calories needed if carbohydrate were provided. This "wear and tear" quota of protein metabolism is intimately related to the carbohydrate metabolism.

In man Landergren (1903), Folin (1905), and Cathcart (1907) found that the protein metabolism could be reduced to one third the fasting value by the addition of carbohydrate.

(3) By replacing all the carbohydrate or fat, or a definite proportion thereof, by the other principle in a diet of maintenance.

Kayser (1894) compared the efficiency of carbohydrates and fats as sparers of protein by replacing practically all the carbohydrate of his diet of meat, rice, butter, cakes, sugar, oil, vinegar and salad, with fat - the two diets being practically of the same fuel value and protein content. The following table shows the effect on the N balance of feeding isodynamic quantities of carbohydrate and fat.

Day	Intake				Output T.N. g.	N balance g.
	T.N.g.	Fat g.	Carb.g.	Fuel Value.		
1st	21.15	71.1	338.2	2,590	18.66	+2.46
2nd	"	71.8	"	2,596	20.04	+1.11
3rd	"	"	"	"	20.59	+0.56
4th	21.31	"	"	2,600	21.31	0.00
5th	21.51	221.1	none	2,607	23.28	-1.77
6th	21.55	217.0	"	2,570	24.03	-2.48
7th	"	215.5	"	2,556	26.53	-4.98
8th	21.10	70.4	338.2	2,581	21.65	-0.55
9th	"	"	"	"	19.20	+1.89
0th	"	"	"	"	19.65	+1.45

In substituting fat for carbohydrate on the fifth day a arked loss of N occurred which became progressively worse. On eturning to the basal diet with adequate carbohydrate the loss eased and a retention of N occurred despite the fact that the otal calories were unchanged.

In 1903 Landergren pointed out that on an exclusively carbo-ydrate diet the output of N steadily fell, and when the diet was hanged to one composed exclusively of fat the output of total N ose. Cathcart (1909) confirmed and extended this, and in 1922 howed the characteristic influence of small amounts of carbo-ydrate when added to a diet of fat.

(N metabolism on 3rd day of each period).

Diet (3,102 - 2,978)	Carb. Cals. %	N in urine/g.			
		T.N.	Urea	Ammonia	Uric acid
323 g. olive oil	0	14.18	10.44	1.13	0.029
310 g. " " +30g.glucose	3.8	10.23	6.60	0.58	0.066
297 g. " " +60g. "	7.5	8.60	5.86	0.38	0.108
279 g. " " +100g. "	12.8	7.12	4.79	0.18	0.145
257 g. " " +150g. "	19.0	7.37	5.05	0.16	0.143

The output of creatinine was but little affected by the change of diet. Small amounts of creatinine were excreted on the carbohydrate-free diet. The characteristic change was the reduction in urea and ammonia and the increase in uric acid excretion.

Tallquist (1902) compared the effect of partial replacement of carbohydrate by fat in the diet and found this to have only a transitory effect upon the amount of protein metabolised. The subject was Tallquist himself, a man 28 years old, in good health and weighing about 80 kg. The diet contained 36 Cals./kg. and during the first period consisted of meat, milk, butter, bread, sugar, coffee and beer. During the second period there was no change in the nature or amount of the protein, but less sugar and more butter were taken so that about half the carbohydrate was replaced by fat - the total fat representing about one half to a third of the total fuel value of the diet. The change had the immediate but transitory effect of causing an unfavourable

influence upon the N balance for a space of two days. N equilibrium was established on the third day.

Atwater (1904) compared the protein sparing action of carbohydrate and fat in a subject who performed a considerable amount of work. The change from carbohydrate to fat involved some 2,000 Cals. - nearly half the fuel value of the day's diet. The average results per day were as follows:-

	On carb.rich diet.	On fat rich diet.
Available calories in food	4,532	4,524
Heat equiv. of work done	558	554
Daily intake of N	17.5	17.1
Daily output of N (Urine	16.6	18.1
Daily output of N (Faeces	2.5	1.7
N balance	-1.6	-2.7

In this series of experiments there is again a difference in the N balance in favour of carbohydrate, but in this instance a very slight one.

Zeller (1914) studied the protein metabolism of a man whose diet contained very little protein, and found that a mixed diet of carbohydrate and fat was just as efficacious in reducing the consumption of body protein as one of carbohydrate alone, so long as the proportion of carbohydrate to fat did not fall below 1:4. Zeller's experimental periods were very short, being insufficient to allow of the organism adjusting its metabolism.

The fat-rich diet did cause the characteristic marked rise in the output of N both in the dog and in man.

It would appear that the carbohydrate of the food cannot be entirely replaced by an equal number of calories in the form of fat. As Cathcart (1922) has pointed out "isodynamic" (equality of energy) is not the same as "isotamieutic" (sparing equality). The latter term covers all phases of cellular activity. If the replacement of the carbohydrate by fat is such as to affect not more than half of the total calories, the effect is but slight on the N-balance. But if this is exceeded, then an unfavourable influence is exerted on the N balance.

The sparing effect of carbohydrate and fat on the loss of protein which occurs on an insufficient diet is easily explainable, but the fact that on occasion the addition of carbohydrate to a diet already sufficient may cause an actual storage of protein, indicates that this protecting power is "something more than merely the question whether the body "needs" to burn protein as fuel" (Sherman, 1935).

Another aspect of the problem is phlorhizin diabetes. As a result of the very striking relationship of carbohydrate to the excessive protein metabolism of the phlorhizinized dog, it has been said that glucose spares protein, even though the glucose producing the effect is being excreted (Larson and Chaikoff, 1937). This statement requires to be taken with reserve for Wierzuchowski (1926) has pointed out that in such an animal, the administration of glucose was followed by the oxidation of a small quantity of the sugar, and this has been

confirmed by Deuel, Wilson and Milhorat (1927). Therein may be the explanation of the protein-sparing action of carbohydrate in phlorhizin diabetes.

Maignon (1933) has adduced evidence to show that the relative effects of carbohydrate and fat on protein utilisation by the rat are dependent on the ratio of the protein to fat, or protein to carbohydrate in the diet fed. With rations of average protein content (egg albumin 1, lard 2; egg albumin 1, carbohydrates 4.7), the N-balance was approximately the same in both cases; on the other hand with rations containing a high proportion of protein (egg albumin 1, lard 0.5; egg albumin 1, carbohydrates 1) the N-balance was in all seven experiments in favour of fat. When the ration contains a high proportion of fat or carbohydrate: egg albumin 1, fat 3; egg albumin 1, carbohydrate 6-7, carbohydrate may prove to be superior to fat.

In order to determine the rôle played by the general rise in energy value of the diet above the energy requirement in these earlier experiments, Subjects 5 and 6 submitted themselves to a further series designed to elucidate this factor.

EXPERIMENTAL.

In the prosecution of these experiments the procedure adopted was somewhat similar to that used in the previous part. Experiment 1 was carried through in the latter half of April and beginning of May, Experiment 2 in July and Experiment 3 in September, 1936.

Experiment 1. Subject 1 (Subject 5 of previous parts). Table 26, Fig.12.

The basal diet differed from that previously adopted in having no cheese present otherwise the quality was the same and the quantity only slightly different. During an initial period of three days weight was lost slightly and the diet had to be adjusted by incorporating more bread to balance this tendency. The calorie value of the basal dietary was approximately 3400 as calculated from Plimmer's tables (75 g. protein, 130 g. fat and 460 g. carbohydrate). The 'first class' protein moiety amounted to 19 g. of milk proteins. The actual N intake was 15.06 g./day as determined by analysis of the composite diet. The additions of beef, lactose and butter which were added subsequently were equivalent to the protein, carbohydrate and fat content of a litre of milk. Water equivalent to that present in a litre of milk was added to the drinking water, a control observed in all these experiments.

When N equilibrium was established, 160 g. lean topside beef were then added to the diet for a period of five days, then 46 g. lactose and 35 g. butter were superimposed for a further 5-day period and finally, the protein content being kept

TABLE 26.

Subject 1.

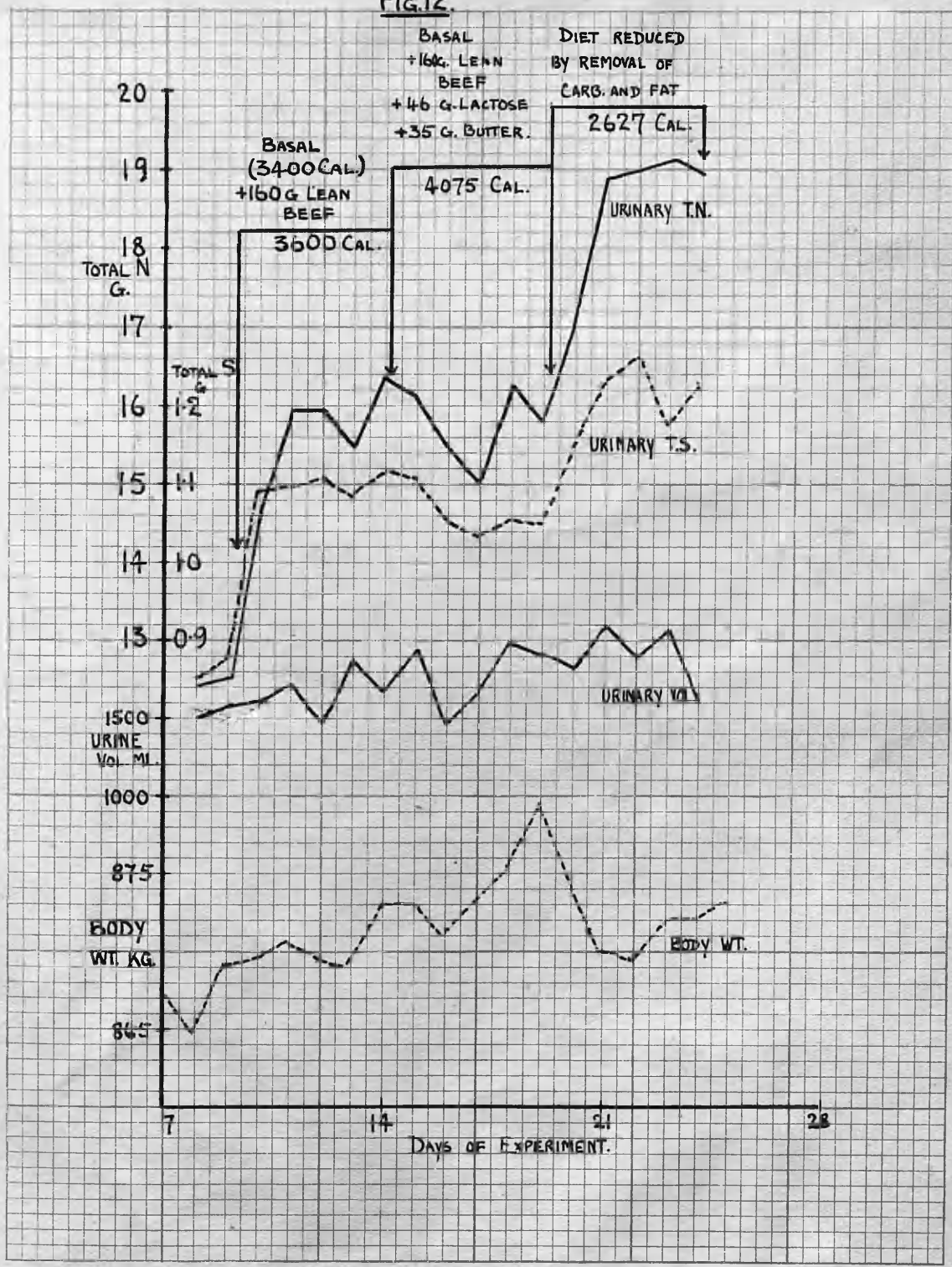
Day of Expt.	Body weight kg.	Daily diet.	Total Cal.	Urine			Av. daily faecal N g.	S.N of retained material
				Vol. ml.	Total N g.	Total S g.		
4	86.70	Basal adjusted to maintain body weight.	3400					
5	86.55							
6	86.15							
7	86.75			1565	12.07			
8	86.45			1500	12.42	0.855	1.765	
9	86.90			1570	12.50	0.880		
10	86.93	Basal + 160 g. lean topside beef	3600	1605	14.68	1.093		
11	87.05			1710	15.95	1.099		
12	86.95			1480	15.92	1.103	1.491	1:33.8
13	86.90			1880	15.45	1.086		
14	87.30			1670	16.35	1.118		
15	87.30	Basal + 160 g. beef + 46 g. lactose + 35 g. butter	4057	1925	16.11	1.114		
16	87.10			1475	15.43	1.049		
17	87.33			1660	15.00	1.034	2.317	1:21.6
18	87.50			1990	16.24	1.058		
19	87.93			1900	15.78	1.044		S:N of material stopped being retained
20	87.42	Basal reduced in carbohydrate and fat but not protein. Ordinary diet.	2627	1810	17.02	1.153		
21	87.00			2100	18.92	1.232		
22	86.95			1800	18.98	1.262		
23	87.19			2040	19.08	1.178		1:19.00
24	87.20			1600	18.95	1.230		
25	87.30							

Rise in body weight at end of 5 days extra beef = 0.40 kg.

Further rise in body weight at end of 5 days extra beef + lactose + butter = 0.63 kg.

Fall in body weight at end of 5 days reduced intake = 0.73 kg.

Fig. 12.


BASAL
+164G. LEAN BEEF
+46 G. LACTOSE
+35 G. BUTTER.
DIET REDUCED BY REMOVAL OF CARB. AND FAT
2627 CAL.
BASAL (3400 CAL.) +160 G LEAN BEEF
4075 CAL.
3600 CAL.
URINARY T.N.
URINARY T.S.
URINARY VOL.
TOTAL N G.
TOTAL S G.
URINE VOL ML.
BODY WT. KG.
BODY WT.
20
19
18
17
16
15
14
13
1·2
1·1
1·0
0·9
1500
1000
875
865
7
14
21
28
DAYS OF EXPERIMENT.

constant, the diet was reduced from 4057 Cal. to 2627 Cal./day for a final period of 5 days by the removal of 900 Cal. fat (butter) 530 Cal. carbohydrate.

The additional food was consumed in two equal portions at lunch and at supper. The N content of the beef was variable (5.4-5.9 g./160 g. beef) but as in the previous experiments with beef an average value was selected in the final computation.

On superimposing beef alone the excretion of total N and S in the urine gradually rose, but even after 5 days it fell distinctly short of the level to be expected if N and S equilibrium had been established. The daily output of N during the first three days was greater than that observed in an earlier experiment in which beef + lactose + butter were superimposed for an equivalent period. This experiment which we have just recalled had to be abandoned owing to illness.

After five days of extra beef intake, the addition of 46 g. lactose and 35 g. butter caused a depression of N and S elimination which tended to reach a constant value after three days, i.e. the existing retentions of N and S were still further increased. The increment in the energy value of the diet caused a still greater saving of protein, or it may be that preferential oxidation of carbohydrate and fat had delayed the catabolism of the retained N and S compounds - presumably protein (Table 26).

As the course of N excretion appeared now to be following the same type of curve noted with Subject 6 of previous paper when he consumed extra beef, lactose and butter over a period of 15 days, it was decided to withdraw sufficient non-protein cal-

ories to bring the total intake to a value below the subject's actual requirement at that metabolic plane. Food equivalent to 1430 Cal. and composed of butter, lactose, cane sugar and apple jelly were removed from the diet. Body weight, which had been increasing during the addition of the beef and more rapidly still under the influence of the additional carbohydrate and fat, now fell rapidly and coincidently the N and S excretions rapidly rose so that after two days, in place of a very definite daily retention of N and S, there was now a very small loss which continued for the few remaining days of the experiment; the elimination of the retained N and S material taking place at an even slow rate and distinct from the more rapid catabolism of the other retained materials presumably mainly fat, as evidenced by the initial rapid fall in weight. It was apparent that immediately the necessity to get rid of superfluous food material was removed the normal intake of protein could be dealt with, and that the stored material was slowly got rid of at a relatively uniform rate. This last observation may help to explain the fact that in the somewhat similar experiment on this Subject 6 in the preceding paper, the basal level of N excretion was not reached following the resumption of the basal diet. In this case there was also a slight but steady loss of N.

The reduction in the calorie value from 4057 to 2627 Cal. was drastic, but when the latter diet was consumed over a period of five days the fall in body weight had not reached the basal value. Indeed it tended to remain constant after falling for the first two days, suggesting that a new metabolic plane had been reached.

The S:N ratio of the material retained during the first 5 days of additional intake was 1:33.8 and during the period of extra carbohydrate and fat 1:21.6, the S:N ratio of the beef being 1:18.55. The values for the retained material are comparable to the recalculated values obtained from Wilson's data. No account was taken of the faecal loss in these calculations. To calculate the S:N ratio of the material which had ceased being retained, the total urinary outputs of N and S for the preceding 5-day period of beef + lactose + butter were determined, viz. - 78.56 g. N and 5.299 g. S. These values were deducted from the total urinary N and S excretions on the low calorie diet, viz. - 92.95 g. N and 6.055 g. S. The S:N ratio of the material stopped being excreted was therefore 1:19.0, a value identical to the S:N ration of the beef ingested and quite distinct from the S:N of the total output for this final period which was 1:15.35.

The increase in weight during the period of overfeeding was approximately 1.03 kg., and the retention of N, 24.89 g. On the basis of 1 kg. body substance (i.e. mainly muscle) equivalent to 30 g.N the expected increase would have been 0.83 kg. Considering the many factors which affect body weight and the doubtful generality of Rubner's basis, there appears to be a fair correspondence again between the calculated and actual gain in weight in this and other experiments. This correspondence is probably more apparent than real.

This experiment confirmed our growing belief that the retention phenomena which we had observed in the milk, beef and soya bean experiments described in the previous parts, were due

to the protein 'saving' effect of the excess energy content of the diet, for here we have found that when protein and/or carbohydrate and fat are added to the diet, so that the total energy value of the diet is in excess of the organism's requirement, N and S storage take place - at least for a time. When the caloric value is cut down below maintenance or probably even to maintenance requirement, the storage rapidly ceases. There is probably then a slow elimination of the stored N and S.

We have purposely used the term protein 'saving' in place of protein 'sparing', for in these experiments there is a direct banking of material, presumably protein.

The next question which required to be determined was an assessment of the relative effectiveness of carbohydrate and fat as protein savers.

Experiments 2 and 3. Subjects 1 and 2 (5 and 6 of previous parts). Figs. 13 and 14, Tables 27 and 28.

A preliminary experiment was carried out by Subject 1, the basal diet being similar to that used in previous experiments with the exception that the milk was reduced to 240 ml./day and slight alterations made in the bread intake. The diet was calculated to have an energy value of approximately 3200 Cal. (protein 66 g. - 9 g. first class; fat 113 g. and carbohydrate 460 g.). Body weight was maintained at a constant level for a week prior to the superimposition of the extra food. When a constant urinary N excretion was achieved carbohydrate equivalent to 780 Cal. were added, the addition being made in the form of 'lemon sago' composed of sago, sugar, syrup and lemon juice. This pudding was divided into two equal portions and consumed at lunch and supper. Some difficulty was experienced in consuming this material on an already satisfying diet. The superimposition of additional carbohydrate effected a saving of 3.68 g. N in the 3-day period and, on renewing it, retention ceased there being a lag of a day before the basal value was reached and apparently passed. It appeared as if there was a slow liberation of the previously stored N.

On the third day of resumed basal, additional fat in the form of 90 g. fresh butter - 700 Cal. - was added. This was easily taken and caused a definite but less marked sparing action. Owing to a limitation of time for the conduct of this experiment, it was not possible to prolong the basal period preceding the addition of fat. Strictly speaking time should have

Table 27.

Subject I.

N and S saving effect of superimposed carbohydrate and fat on a diet adequate for maintenance.

Day of expt.	Body weight kg.	Daily diet.	Total Cal.	Urine			Av. daily faecal N g.
				Vol. ml.	Total N g.	Total S g.	
0	87.55	Ordinary					
1	87.15	Basal	3200				
2	87.00	"					
3	87.15	"					
4	87.28	"					
5	87.10	"		2020	11.53	0.822	
6	87.00	"		1490	10.96	0.797	2.000
7	87.12	"		1400	10.99	0.753	
8	87.11	Basal + carb.	3980	2055	10.13	-*	
9	87.65	" "		1855	9.56	0.747	2.723
10	87.40	" "		2770	9.57	0.743	
11	87.32	Basal	3200	1830	10.02	0.744	
12	87.08	"		2020	11.36	0.801	
13	87.05	"		1660	11.43	0.814	
14	87.40	Basal + fat.	3900	1580	11.61	0.788	
15	87.60	" "		1770	11.07	0.730	1.467
16	87.48	" "		2540	10.88	0.763	
17	87.15	Basal	3200	1920	10.72	0.753	
18	87.43	"		2230	11.42	0.774	

* Urine accidentally contaminated with H_2SO_4.

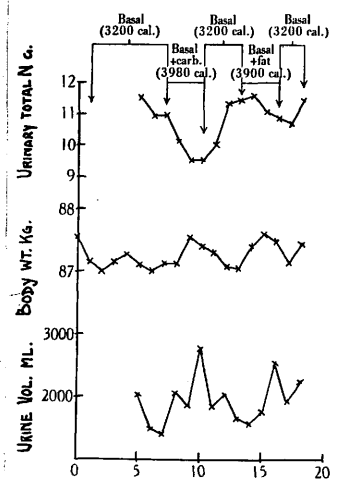


FIG. 13. SUBJECT I. DAYS OF EXPERIMET.

been allowed for a return to the basal N excretion. It is perhaps legitimate to take the average N excretion of the last two days of this second basal period as the base line for the assessment of the N-saving effect of the extra fat, rather than the average basal value of the first period. If this be taken, the retention within the three days of extra fat intake was 0.64 g. N. This saving effect of fat appeared to persist into the first day of the final basal period. That is, there was a lag before basal conditions were again reached. If we take the four days of N retention in each period we find that carbohydrate equivalent to 780 Cal. spared 4.64 g. N (0.59 g. per 100 Cal.) and fat equivalent to 700 Cal. spared 1.48 g. N (0.21 g. per 100 Cal.). The ratio of the $\frac{\text{N saved by carbohydrate}}{\text{N saved by fat}}$ per 100 Cal. was 2.81.

Owing to the accidental contamination of one of the day's collections of urine with H_2SO_4, the relative significance of the S retentions cannot be assessed accurately. The data which are available, unsatisfactory as they are, indicate that the S-saving effects of carbohydrate and fat were not so widely different when assessed over this relatively short period. The retentions of N and S in each period of surfeit feeding were accompanied by increments in body weight, which were in part reduced by a diuresis which occurred in each instance on the third day of extra food.

To rule out the possibility that this difference in N-saving power might be due to the carbohydrate effect not having passed off before the superimposition of the additional fat, the experiment was repeated by Subject 2 but the order of superimposition was reversed.

Subject 2's basal dietary had an energy value of approximately 2890 Cal. (protein 86 g. - 22 g. first class; fat 140 g. and carbohydrate 320 g.), the ingredients being similar in quality and approximately in quantity to that selected in previous dietaries. No reduction in milk intake was made in this case. The additional fat was butter but the carbohydrate selected glucose. The additional butter was all taken at breakfast and to simulate the slow digestion and absorption of fat, the additional glucose was taken in small lots hourly over a period of three hours. The total daily intake of water was kept constant. The addition of 90 g. butter raised the energy value of the diet from 2890 Cal. to 3590 Cal. and caused a definite retention of N and S amounting to 6.42 g. and 0.459 g. respectively. The S:N ratio of this stored material was 1:14.0. Resumption of basal conditions did not cause an immediate return to normal. There was a lag of three days before the normal levels of urinary N and S were attained. The S:N ratio of the material stored during this period was 1:11.6. Although marked by initial fluctuations in body weight this retention was accompanied by an increase in weight, for on resumption of basal conditions a slight fall in weight resulted.

By an error, in Experiments 2 and 3 in place of an isocaloric equivalent of sugar, slightly larger supplements were given (approximately 12 per cent.). In Experiment 3 in place of 185 g. glucose, 210 g. were superimposed for five days, an addition of 780 Cal./day. A greater N and S-saving effect was noted with glucose than with butter even when allowance was made for the slight difference in calorie value. The N-saving effect of

TABLE 28.

Subject 2.

N saving effect of superimposed fat and carbohydrate on a diet adequate for maintenance.

Day of expt.	Body weight kg.	Daily diet.	Total Cal.	Urine			Av. faecal N g.	S:N material saved.
				Vol. ml.	T.N. g.	T.S. g.		
3	63.30	Basal	2890	2257	10.86	-	0.84	
4	62.95			2040	11.01	0.772		
5	63.25			1810	11.06	0.821		
6	63.45			1700	10.55	0.766		
				Mean	10.87	0.786		
7	63.45	Basal + fat	3590	1450	10.01	0.719	1.06	1:14.0
8	63.40			1665	10.25	0.749		
9	63.40			1290	9.78	0.724		
10	63.30			1327	9.73	0.723		
11	63.40			1168	9.49	0.697		
12	63.45			1830	9.54	0.645		
13	63.30	Basal	2890	1880	9.67	0.693	1.03	1:11.6
14	63.30			1410	9.81	0.704		
15	63.05			1630	10.00	0.724		
16	63.15			1175	10.52	0.784		
17	63.15			1210	10.56	0.752		
18	63.15			1435	11.35	0.774		
19	63.25	Basal + carb.	3670	1840	10.08	0.694		1:14.2
20	63.60			1400	9.41	0.719		
21	63.80			1735	9.20	0.668		
22	63.65			1500	8.57	0.629		
23	64.15			1640	8.69	0.630		
24	64.65	Basal + double previous carb.	4450	1860	8.18	0.540	1.13	1:13.0
25	64.80			1825	7.08	0.532		
26	64.85			1730	7.15	0.499		
27	64.10	Basal	2890	2230	8.27	0.643		1:13.8
28	64.20			1690	9.19	0.664		
29	63.95			1980	10.14	0.701		
30	63.85			1820	10.11	0.730		
31	63.15			2040	9.74	0.796		
32	63.90			1885	11.16	0.765		
33	-			1950	10.95	0.729		

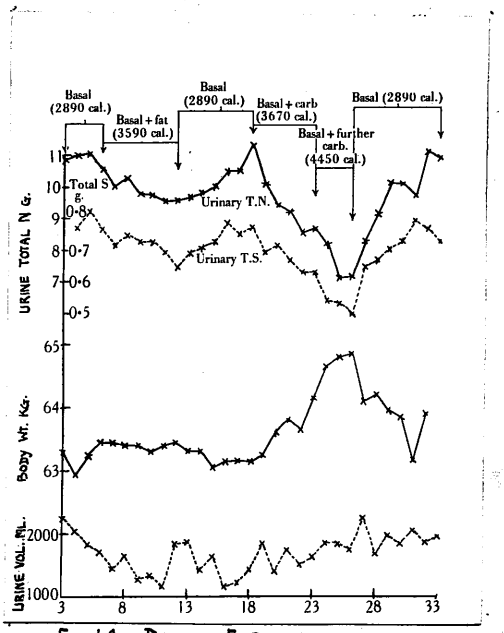


FIG. 14. DAYS OF EXPERIMENT.

the carbohydrate over 5 days was 8.40 g. (1.08 g./100 Cal.), that of fat for an equivalent period 5.09 (0.73 g./100 Cal.). The S-saving effect of the carbohydrate for the same period was 0.59 g. (0.076 g./100 Cal.) that of fat 0.318 g. (0.045 g./100 Cal.). The ratio $\frac{\text{N/or S saved by carbohydrate}}{\text{N/or S saved by fat}}$ per 100 Cal. was for N 1.48 and for S 1.69 for the period under consideration. Corresponding to this increased power of carbohydrate over fat to save N and S, was its faculty of causing a much greater weight increase. The S:N ratios of the material retained was 1:14.2 - a value similar to that noted during the addition of fat.

At the end of five days the intake of glucose was doubled thus raising the total calorie value of the diet to 4450 Cal., a value far in excess of the subject's requirement, for no additional energy expenditure was incurred. The additional glucose was taken at hourly intervals between 2 p.m. and 6 p.m. and was continued for three days. A further saving of N and S was effected, amounting to 10.20 g. N and 0.787 g. S for the period. The first addition of glucose depressed the urinary excretion of N and S to levels 2.18 g. and 0.156 g. respectively below their basal outputs. The second addition reduced the levels of N and S excretion to values 3.72 g. and 0.287 g. below their respective basals. This second addition of glucose caused the body weight to rise still higher. At the end of this period it had risen by about 1.7 kg. The S:N ratio of the stored material was 1:13.0 at this stage. It appeared possible to spare respectively 34 per cent. and 36 per cent. of the previously metabolised N and S.

Resumption of basal conditions caused a rise in N and S output and a fall in body weight, the basal values being reached in about five days. This rapid decline in weight was not accompanied by a contemporaneous liberation of the stored N and S, nor was it mainly due to the freeing of water from the organism. The material catabolised by the organism during this period must probably have been largely fat. The experiment unfortunately could not be continued longer so that it was not possible to determine the fate of the retained N and S. In view of our previous experiments it is highly probable that these would have been slowly liberated.

It is of interest, but perhaps scarcely legitimate, to compare the N retentions witnessed with Subject 2 (Subject 6 of previous parts) when milk, beef + lactose + butter, glucose and fat were separately superimposed. Per 100 Cal. additional food, the respective values for the N retained during the first 5 days of superimposition were 1.75, 1.62, 1.08 and 0.73. Of course the total protein content of the diets differed during the periods of superimposition.

Discussion.

The sparing effect of carbohydrate and fat on the loss of protein which occurs on an insufficient diet is understandable, but the fact that an increment in the energy value of the food beyond the needs of the organism also causes a retention of N and S, requires an extension of our conception of the metabolism of protein, carbohydrate and fat.

In the review of the literature which prefaced the previous parts attention was drawn to the fact that scattered throughout the literature are to be found occasional records of experiences similar to our own. These have largely been forgotten and only the persistent storage which occurs (1) in the growing organism (or in pregnancy), or (2) where increased muscular exercise results in enlargement of the muscles, or (3) where owing to previous insufficient food or to wasting disease the protein reserves have been depleted, has been studied intensively.

When the adequately fed organism is confronted with the necessity of disposing of food ingested in excess of its energy requirement, it burns what it can and stores what it cannot. An alimentary glycosuria may result if huge excesses of carbohydrate are given. No glycosuria was noted in our experiments. Although observations on the basal metabolic rate during the earlier milk experiments on Subject 2 indicated no elevation of the basal metabolism this cannot be taken as indicative of the absence of an increment in the oxidative processes over the normal during

the twenty four hours. If 24 hour metabolic observations can be made the present writers believe that such work will reveal a definite increase in the oxidative processes, but one insufficient to account for the disposal of all the excess potential energy of the food.

With regard to the storage mechanism we know definitely that it is associated with a retention of N and S and by an increment in body weight. The retention of N in the experiments in which there was a direct increase in the protein intake corresponded approximately to the increment in body weight, if it is assumed that 30 g. N are equivalent to 1 kg. body substance (i.e. mainly muscle (Rubner's basis). This equivalence may not have any real value and did not hold in the last experiment when the supplements were non-protein. We believe that the optimum ratio for the proximate principles in an adult dietary are precisely those selected by the mass of the people, viz. 10 per cent. of the energy requirement from protein (approx. 1 g. per kg. body weight) 25-35 per cent. from fat and the remainder from carbohydrate. If, and only if, the total energy requirement of the organism be exceeded and the substance in excess of its normal content be protein, storage of some of this protein takes place. In the case of protein there is good evidence to indicate that the metabolic plane is gradually raised so that after a time further storage of N ceases. On the other hand, if the substance in excess be carbohydrate the glycogen stores of the liver, muscle and other tissues are soon filled up for the capacity to store glycogen is limited. With

a continued flooding of the glucose into the blood, carbohydrate oxidation rises more and more, while the rate of glycogen deposition falls off. It is at this point probably that fat formation must be set up in order to dispose of the continued supply of carbohydrate. The capacity for fat storage is enormous. With the rise in carbohydrate oxidation and the necessity for its disposal there occurs a diminished deamination of amino acids for the non-protein moiety is no longer required for fuel purposes, consequently there occurs N and also S retention, the unrequired amino acids being stored as new protein or attached as Roche (1933) would have us believe to already existing protein. When it is the fat moiety which is increased, there occurs a rise in the rate of fat oxidation and storage also readily occurs. Again there is not the same demand for protein for energy purposes so that deamination diminishes and storage of the amino acids takes place. In this case however, the effect on protein metabolism is not so marked, perhaps due chiefly to the facility for fat storage and also because the increase in fat oxidation will require for its completion an increment in carbohydrate metabolism and so necessitate the utilisation of more of the non-N residue of the amino acids.

The superimposition experiment overcomes the difficulty of measuring the cutaneous losses, for it can reasonably be assumed that such alteration as may occur in the amount of N excreted by this path on changing the diet will not alter appreciably the relationship between excess intake and excess

output. This factor and the question of defective digestion and absorption from the gut can be ruled out of account as contributing to the observed phenomena.

The element of time plays a part in this process. Larson and Chaikoff (1937) have recently performed experiments on the dog which have an intimate bearing on the mechanism whereby carbohydrate spares N in the normal nutritive state of the dog. In their experiments glucose was added as a supplement to the mixed diet devised by Cowgill. The energy value of the basal food intake was 60 Cal./kg. and was fed once a day. It was found that the single administration of 50 g. extra carbohydrate spared N only when ingested at a definite time relation to the ingestion of the protein of the basal diet. The interval during which the extra carbohydrate is able to exert a N-sparing action is limited to 4 hours before and 4 hours after the ingestion of the daily meal. The most pronounced sparing of N occurred when the extra carbohydrate was ingested at the same time as the meal or within one hour of it. It is believed that it is the N to which the protein of the diet contributes which is affected by the additional glucose. The decreased N excretion did not affect the whole 24-hour period. In Larson and Chaikoff's study it was observed during the first 12 hours following the carbohydrate feeding. The N eliminated in the next 12 hours had already begun to show a rise above the previously established equilibrium level. They conclude that the N spared under the influence of a single feeding of extra carbohydrate is not permanent, its elimination beginning several hours after its storage is effected.

Repeated daily additions of extra carbohydrate at the same time that protein is ingested result in N retention, but this continues only as long as the daily administration of extra glucose are continued, and the effect only takes place during the first few hours following the carbohydrate addition. When the extra glucose is discontinued an increased outpouring of N takes place in the interval corresponding to that in which N storage was observed.

Although the increase in calorie intake in the majority of our experiments was some 25 per cent. it proved about the limit for comfort during protracted experiments where the energy expenditure was maintained at a relatively constant level. It is well known that some people easily 'lay on' fat whilst others remain spare despite attempts to gain weight. The difference probably lies in a dissimilarity in capacity to raise the total metabolism to meet the excess energy input. On the other hand it is frequently found that normal individuals who state that they cannot fatten themselves are guilty of self deception.

It is fairly obvious that the processes of retention which occur in the normal organism faced with an ingestion of excess food cannot go on indefinitely. In the absence of a rise in the metabolic plane sufficient to utilise the excess energy, the condition which limits the process is simply failure of appetite. The normal adult organism as soon as possible catabolises the stored material as evidenced by the rapid reduction in weight which occurs when the additional intake is removed. This is not so certain in the later stages of adoles-

cence for further growth is still possible, and it is of interest that the S:N ratio of the material stored closely resembles that lost in starvation.

Lack of appetite, as a factor limiting the process of overfeeding, was demonstrated in an interesting fashion by some observations on another subject not previously described in this series. During a prolonged period of daily observation of his weight, this subject attempted to superimpose 500 ml. raw milk per day without, at least consciously, reducing his usual diet. Body weight did not rise but fell. An independent observer of this experiment noted that the subject had unconsciously reduced his intake. This was substantiated by the fact that in a previous trial with 1000 ml. additional milk per day, the intake of other food had obviously had to be reduced.

Our observations may shed some light on the effect of work on protein metabolism. If the energy intake is in excess and the work done insufficient to utilise the excess, a rise in the output of N would be expected, a rise greater than that due to a general increment in 'wear and tear'. On the other hand, if work is superimposed on a dietary which is just adequate to meet the new energy requirement, then no rise beyond perhaps a slight increment due to wear and tear would be anticipated. Experiments to elucidate the effect of work are in process of being formulated.

It is of interest to consider the possible nature and site of this retained N and S.

The nature and site of the retained N.

1(a). Retention or elimination of end products of metabolism.

Terroine (1933) has pointed out that in the case of Voit's dog, whose reaction to variations in the protein intake has already been described, the retention of N during the lag period following the increase in intake was 23 g. equivalent to 53 g. urea in the case of this 30 kg. animal. The total urea content of a 23 kg. animal richly fed with meat was found by Schöndorff (1897) to be 32 g. It is unlikely if not impossible that the N material retained could have been urea. This is borne out by the experiment of Voit in which he found that urea added to a diet was immediately and completely rejected.

1(b). Retention or elimination of intermediary metabolic products.

Whilst the retention of N as ammonia in any quantity is unlikely, its retention as polypeptides or amino-acids would appear possible since we know that amino-acids and certain peptides may increase in the blood in the course of their absorption from the intestine and disappear rapidly. However, it is well established that the free amino-acid N in the tissues remains constant despite wide variations in diet and nutritional state. The observations of Van Slyke and Meyer (1913-14) and Wishart (1915) indicate that six hours after a protein meal the free amino-N of the tissues is at the fasting level. This is irrespective of the fact that the urinary N does not return to the fasting level for some hours after a large meat meal (Williams, Riche and Lusk, 1912). The following table has been taken from Van Slyke and Meyer's paper.

Condition.	Amino-N in mg. per 100 g. tissue.					
	Liver	Kidney	Spleen	Triceps	Gracilis	Pancreas
Dog receiving 500 g.beef daily for 7 days in addition to ordinary diet.	59	56	92	58	51	61
Dog starving for 2 days.	69	45	69	64	53	55

While these values do not indicate an increased concentration there might have been a total quantitative change for the total weight of the organ or muscle would probably have been larger in the well-fed animal. The work of Mitchell, Nevens and Kendall (1922) answers this point. They compared the total non-protein N of all the tissues of animals receiving a normal diet with those receiving a protein-free diet and found no real difference.

Van Slyke and Meyer (1913-14) were unable to find any increase in polypeptide N in the tissues of the dog after a large intravenous injection of amino-acids. They deduced that the retained amino-acids are not stored as such but are converted into protein. The difficulties that have been raised in the way of accepting this hypothesis will be recounted later.

2. New formation or destruction of tissue.

Pugliese (1904) compared the relationship of the liver to the rest of the animal (dog) in the nourished and starved states and in the subsequent period of realimentation.

Wt. of dog (kg.)	6.2	4.6	3.4	3.8	5.5
State	Fed	Starved	Starved	Refed	Refed
Wt. of liver	210	95	70	210	280
N of total liver	5.73	3.23	2.53	4.21	5.40
g. liver/kg. body wt.	33.8	21.1	20.5	55.2	50.9
g. N of liver/kg. body wt.	0.924	0.716	0.713	1.10	0.981

These observations demonstrated that the liver was extensible, increasing in weight during feeding, decreasing during fasting. A very striking demonstration of this was provided by Gautier and Thiers' (1928) experiments on the partial ablation of the liver of frogs. After 8-10 days' fasting the ratio of $\frac{\text{total weight of liver}}{\text{weight of right lobe}}$ had a value of 1.42, 1.91, 1.39 and 1.35 in the four frogs examined. From another group the right lobe was removed and the animals fed cheese. The ratio of $\frac{\text{total weight of liver}}{\text{weight of right lobe (previously removed)}}$ was 5.34, 4.95 and 3.97. These changes were paralleled by similar changes in the N content of the livers.

Gautier (1929) also noted that the liver tissue of the frog increased markedly before hibernation but that the percentage increase in total proteins fell short of the percentage increase in weight.

	Wt. of liver.	Wt. of total liver proteins.
June (av.of 4)	0.7668	0.1368
Sept.(av.of 7)	1.3325	0.1819

Is the retention in the form of protein material?

This is a very old question and a considerable amount of work has been done in an attempt to elucidate the problem.

As Terroine (1933) has pointed out, the essential character of a reserve is its variability. His work in particular, has shown that the organism possesses two great groups of substances:-

(1) The necessary elements of all the tissues in equilibrium amongst themselves and presenting fixed ratios one to the other; the concentration of which rather than their nature is not modified by any external influence; and

(2) Reserve elements, variable in quantity and quality and whose presence effects necessary differences in the total composition of the organism.

It is well established that one can increase or diminish the amount of fat and glycogen by an appropriate dietary régime and on this is based the proof of the existence of fat and carbohydrate reserves. In the same manner the N content of the organism ought to consist of a fixed element which participates in the con-

stitution of the tissues and a variable element which increases or decreases according to the metabolic state. This would correspond to the "constant" element and "variable" element which Terroine (1920) established for fatty acids.

As Terroine points out, if there be a reserve of protein then:-

(1) Members of the same species taken at random and presenting different net values for the total fatty acids and glycogen ought to vary in their N content; and

(2) The proportion of the N contained in the organism should be higher the greater the amount of protein of the diet.

(a) <u>The N content of the whole organism</u>.

Terroine, Brenckmann and Feuerbach (1924) found no important differences in the N content of the carcases of mice and of a small bird (Vidua principalis). Such differences as occurred were apparently due to changes in the amount of fat and disappeared completely in the starving animal. These authors then proceeded to try out the effect of different dietary conditions and obtained the apparently paradoxical result, that the starving subjects were richer in N than the others. This was due to the fact that the disappearance of the fat reserves caused a relative increase in the percentage of protein present. This particular type of experiment has been repeatedly confirmed. For example Chanutin (1930) found the N content of the rat unmodified by the repeated ingestion of rations rich in protein.

These facts cannot be taken as evidence against the existence

of a protein reserve. Terroine and Ritter (1927) thought that a relationship might exist between the magnitude of a reserve and the imminence and need which it might have to fulfil. It is well known that the N wastage through 'wear' and 'tear' is very little and even if one ceases to cover it for a fairly long time little real damage is done to the organism, so that it is not surprising if the storage of nitrogenous material as a true reserve is insignificant or, if limited in extent, is dwarfed in the analysis of the total carcase.

(b) The N content of the tissues.

With regard to the total N content of specific tissues the evidence is equivocal. In 1905 Cathcart and Leathes showed that during active absorption from the intestine there was a definite amount of storing of nitrogenous material in the liver. The next year Seitz (1906) demonstrated that storage of protein could take place within the hepatic cells of birds. Observations such as those of Gautier and Thiers (already reported), Tichmeneff (1914) and Rothmann (1924) indicate that the livers of starving animals have a higher percentage of N than those fed on normal or protein rich diets. On the other hand, Greene noted that there was a fall in the protein content of the fasting salmon and that this fall was accompanied by an increase in the water content of the muscle. In their comparison of fat and emaciated oxen, Hoagland and Powick (1925) found in the former a slightly greater content of protein material in the muscle and a slight diminution in its water content.

State.	% Water.	% Protein.
Extremely emaciated oxen	80.45	18.94
Thin oxen	79.38	19.80
Fat oxen	76.27	22.09

On the other hand Moulton (1920) could find no evidence which indicated that undernourishment rendered the muscle of the ox more watery.

Luck (1936) has recently shown that mammalian liver yields four fractions, viz. globulin II, euglobulin, pseudo-globulin, and albumin and in rats maintained on a low protein diet these were respectively 5.07, 4.58, 1.06 and 0.86 g. per 100 g. liver. On a high protein diet each of the above fractions was increased by 50-60 per cent. There was not only hyperplasia or hypertrophy but also an increased content of protein per unit of tissue. All the liver proteins therefore participate equally in the function of storage. Addis, Poo and Lew (1936, 1,2) have investigated the quantities of protein lost by the various organs and tissues of rats during a 2-day and 7-day fast. After a 2-day fast the livers lost 20 per cent. of their original protein content, the kidney, heart and all the other organs and tissues combined lost 4 per cent. During a 7-day fast the liver lost 40 per cent. of its protein, the prostate and seminal vesicles 29 per cent., the heart, kidneys, drawn blood and alimentary

tract 18 to 28 per cent., the muscle, skin and skeleton 8 per cent. and the brain 5 per cent. Of the total protein lost in 7 days the muscle, skin and skeleton contributed 62 per cent., the liver 16 per cent.

At this point it may be of interest to consider the nature of the material stored during the fattening of mature domesticated animals.

It is natural to expect that the main gain should be fat and this has been demonstrated in two main ways, viz. (a) by determining the nature of the difference in the analyses of the carcases of lean and fattened animals, and (b) By means of balance experiments determining the composition of the organic matter gained.

(a) Comparative slaughter tests.

The classical experiments of Lawes and Gilbert (1859) were probably the earliest examples of this method of computing the composition of material gained during fattening of domesticated animals. Unfortunately only a certain number of their beasts were

mature, the rest only partially so. Armsby (1917) has collected data concerning the increase in live weight of a number of beasts and his table in a modified form is given below along with the observers' names.

Animal.	Av.age years.	Composition of increase.			
		Water %	Ash %	Protein %	Fat %
Cattle (Lawes & Gilbert)	4	24.64	1.47	7.69	66.20
Sheep (Hemelberg, Kern and Wattenberg)					
Fat	$2\frac{3}{4}$	25.80		6.64	67.56
Very fat	"	20.30		5.23	74.47
Last stage of fattening	"	6.45		1.68	91.87
Sheep (Friske)	4	12.03		15.07	72.90
Sheep (Pfeiffer & Friske)	$3\frac{1}{2}$	64.33		7.11	28.56

(b) Balance experiments.

Again we are indebted to Armsby for summarising the available data - data often buried in little known agricultural journals. The table below is essentially his, though modified in certain respects.

Animal.	Av. composition of organic matter.	
	Total protein %	Fat %
Cattle (Kellner)		
Group 1	26.25	73.75
" 2	23.30	76.70
" 3	17.17	82.83
" 4	12.55	87.45
" 5	8.06	91.94
Sheep (Henneberg, Fleischer and Müller)	4.26	95.74
Swine (Meissl, Strohmer and Lorenz)		
Animal 1	9.75	90.25
" 2	10.67	89.33
" 3	16.39	83.61
" 4	15.16	84.84

During the process of fattening there is also a gain of more or less protein and small amounts of mineral matter in addition to the fat. In Kellner's experiments in particular, the gain in protein was quite considerable. A certain amount of this gain must be accounted for by growth of the epidermis and its appendages. A further amount of this protein is to be found in the subcutaneous adipose tissue itself for there appears to be an actual increase in the number of cells and these contain protein. Armsby calculates that a kilogram of subcutaneous adi-

pose tissue of average composition would be equivalent to the storage of about 45 g. of protein equivalent to 7.2 g. N. It is doubtful if the epidermal and adipose tissue can account for all the N stored during the process of fattening. Some other site or sites for its deposition must be sought.

In a very interesting investigation Henneberg, Kern and Wattenberg (1878) computed the composition of the meat of their thin and very fat sheep on a fat-free basis:-

Fat-free meat of sheep.

Fraction.	Thin sheep.		Very fat sheep.	
Water		79.41		79.02
Insol. protein		15.85		15.73
Extractives				
Sol. protein	1.29		1.93	
Non-protein	2.18		2.17	
Ash	1.27	4.74	1.15	5.25
Total		100.00		100.00

The actual gains were:-

Insol. protein		38.7 g.
Extractives:		
Sol.protein	82.2 g.	
Non.protein	4.0 g.	
Ash	-9.2 g.	
		77.0 g.
Total		115.7 g.

These observations indicate that during fattening the muscles gain in protein and that the gain involves the insoluble and soluble fractions. A small fraction of this increase in muscle protein may be located in the cells of the intramuscular fat layers for there is a marked increase of this material during fattening. The so-called "marbling" is due to this extensive increase in intramuscular fat.

From these experiments it is apparent that no general deduction can be made as to the behaviour of the total N of any particular tissue during different dietary régimes.

(3) The constitution of the total protein material of the tissues.

If the N is stored in the tissues as protein then it is not impossible that the constitution of the total protein should change, for it is unlikely that the stored material should necessarily possess the same molecular structure as the proteins entering into the active protoplasmic mass of the tissue. Cahn and Bonot (1928) analysed the muscles of normal and fasting rabbits and dogs for arginine and cystine, without detecting any significant difference. On the other hand, Roche (1933, 1) found in rats that the N loss on a N-free diet came from partial disintegration of the molecules of muscular protein, substantiating MacCollum and Steenbock's earlier hypothesis (1912). The mono-amino acids increased, the di-amino acids greatly decreased; cystine, histidine and arginine remained constant, while tryptophane, tyrosine and lysine diminished (Roche 1934, 2). In the absence

of food protein, the N requirement was met by the partial mobilisation of muscle protein and not of a special reserve protein (1934, 1). Histological observations by Roche (1933, 2) were made on the muscles of rats fed a protein-free diet till they died. She found a decrease in the diameter of the muscle fibres in comparison with controls, while the number of nuclei per fibre was almost doubled. No signs of degeneration were noted. The water soluble N of the muscle protein increased by about 21 per cent. In complete starvation this increase was of the order of 39 per cent. This fraction she considered as intermediary in the equilibrium:-

Circulating N $\leftrightarrow$ extractable muscle N $\leftrightarrow$ muscle protein N.

Can we extend this idea to the question of retention or loss of N in the period preceding the establishment of a new equilibrium? Is it also to be explained by the integration or disintegration of existing protein units? In this process does liver, in particular, but also muscle tissue play the dominant rôles? The protein reserves of the organism are relatively enormous and if they are capable of waxing and waning in molecular complexity even to a very slight extent, N retention and N loss become explicable. Rubner (1902), and Atkinson and Lusk (1919) have proved that the fraction of the ingested protein which is absorbed and retained exercises no specific dynamic action. A fact which indicates that this deposited N is for a time in a particularly stable state.

It is interesting to compare this most recent theory

with the older hypotheses. Liebig was probably the first to put forward an account of the transformations of food protein into tissue protein. At that time it was presumed that food protein underwent but little change during digestion. Liebig's theory became discredited as soon as it was realized that food protein was hydrolysed into its constituent amino acids during digestion. The theory of Voit (1867) which supplanted Liebig's enjoyed wide acceptance until it was subjected to a searching criticism by Pflüger.

Voit held that the protein after absorption circulated in the tissue fluids, and was catabolized under the influence of the living protoplasm but without first becoming an integral part of it. This 'circulating protein' replaced the wear and tear of the resistant 'tissue' protein, there being no chemical difference between them. Pflüger, on the other hand, believed that there was a decided difference in the so called circulating protein and the tissue protein - the former being relatively stable, the latter highly labile and susceptible to oxidation. Pflüger believed that all food protein must first become an integral part of the living protoplasm before being utilized - an extension of Liebig's hypothesis. Kassowitz (1904) has largely subscribed to this.

Rubner (1908) believed in a transitional type of protein analagous to Voit's 'circulating protein' and in a 'wear and tear quota'. Apart from that, the greater part of the food protein was rapidly deaminized and the non N-residue oxidised forming the dynamic quota. The theory of Speck (1903) was somewhat similar

but he held that the N-residue was not immediately converted into urea but shared in the formation of a variety of substances. Eventually these substances were disintegrated, the N then being converted into urea.

Folin (1905) took up the Voit-Pflüger controversy and pointed out that Pflüger had not really disproved Voit's theory. With regard to Pflüger's theory, Folin considered it incredible that such an enormous building up of bioplasm accompanied by immediate destruction could take place in such a short time. Folin considered that there are two essentially different forms of protein catabolism, a constant or endogenous one yielding chiefly creatinine and neutral S and to a less extent uric acid and ethereal sulphates, and a variable or exogenous catabolism yielding chiefly urea and inorganic sulphates. The former type represented the tissue metabolism - the decomposition of living protoplasm. Folin considered his exogenous protein metabolism had nothing to do with Voit's circulating protein. He held that only a very small amount of protein was required by the organism - just sufficient to cover the endogenous metabolism. The present writer (1931) has pointed out that in conditions where body protein is being catabolised at a faster rate than normal, the chief representatives of this endogenous catabolism are not creatinine and neutral S but urea and inorganic sulphate. The writer dissociates Folin's terms 'constant' and 'endogenous', 'variable' and 'exogenous' and believes that it is legitimate to consider principally a constant and a variable protein metabolism, at least as far as the interpretation of the end products in the urine are concerned.

McCollum and Steenbock (1912) assumed that the processes of cellular catabolism and repair do not involve the destruction and resynthesis of an entire protein molecule. In the endogenous protein catabolism the destruction of all amino acids is not in proportion to the extent in which the individual amino acids occur in the disintegrating protein molecule. According to Sherman (1935) it is "helpful to think of the protein metabolism not only in terms of building and repair, but also of maintaining the (approximate) dynamic equilibrium which exists between proteins and amino acids in the cells of the animal tissues. Concentration of any of the amino acids into which tissue proteins tend to be hydrolysed may therefore be expected to help in pushing the reaction amino acids $\rightleftharpoons$ protein, towards the right; in other words, any of these amino acids may thus (to some extent) function in the maintenance of body protein, whereas for the synthesis of new protein as in growth, all the amino acids which enter into the structure of tissue proteins would be needed. Hence it is quite reasonable that proteins of different efficiency for growth may show much more nearly equal efficiency in the normal maintenance of adults ..... "

What might have proved a useful line of enquiry on this problem of whether the retained N becomes true living tissue protein or a special variety of deposit protein has been partially exploded by Wilson's (1931) work. It seemed reasonable to argue that the S:N ratio of the material catabolised on changing from a high to a low diet should indicate the nature of the retained

material provided its S:N ratio was different from that of tissue protein. Wilson found that the organism could store protein with varying S:N ratios, the higher the S content the more unstable the retained material.

(4) The partition of the metabolic N of the urine.

Terroine and Boy (1931) considered that if the retention or loss of N in the period which precedes the establishment of a new N equilibrium be due to the construction or destruction of organised tissue, the distribution of the N compounds in the urine ought to be affected. If the elements of the tissues are degraded or integrated in the same proportion as they occur in the tissues then all the N constituents of the metabolic N - the urea, amino acids and ammonia from the protein and from the other components of the tissues, purine bodies and derivatives, creatine and creatinine ought to rise or fall simultaneously. On the other hand if there has been a storage of protein then degradation of this reserve will result in constituents of protein origin. They extended their argument still further and maintained that if this last hypothesis proved correct it should be possible to evaluate quantitatively the magnitude of the reserve by the passage from an adequate diet to a strict carbohydrate one. In this last state there is a constancy of the N waste and the partition of the N constituents of the urine remains fixed and characterises the minimal N metabolism of the organism. Or, in passing from a régime rich in protein to a régime which is poor, the quantities of the various constituents eliminated in excess in the form of protein end products, will provide a measure of the protein in reserve.

It is to be regretted that for their experiments Terroine and Boy used a pig which was still growing and not an adult one. In the first period of normal diet a regular N retention occurred.

In the second period a strict carbohydrate régime was instituted and this resulted in a progressive decrease in N excretion. A definite N output was reached after 4 days, and was maintained for a time with a constant partition of the N catabolites in the urine.

Régime	T.N.	Urea N.	NH_3 N	Amino acid N	Allantoin	Creatinine N	Creatine N
Rich protein regular N retention - aver. 5 days	71.67	62.59	2.07	1.18	3.71	0.84	0.63
Carbohydrate diet regular N diminution							
1st day	22.40	18.00	1.13	0.58	1.35	0.75	0.04
2nd day	8.28	6.03	0.55	0.50	0.40	0.62	0.05
3rd day	7.30	4.90	0.13	0.38	0.44	1.10	0.06
4th day	6.30	3.38	0.16	0.44	0.40	0.91	0.03
Total 4 days:	44.28	32.31	1.97	1.90	2.59	3.38	0.18
Continuation of above. Av. 10 days	4.46	2.13	0.18	0.42	0.48	0.91	0.02

Terroine and Boy calculated the composition of the material excreted as follows:-

	T.N.	Urea-N+ NH_3-N+ Amino acid-N	Allantoin N	Creatinine + Creatine N
Observed excretion during 4 days' decreasing N excretion	44.28	36.18	2.59	3.56
Calculated endogenous level for 4 days	17.84	10.92	1.92	3.72
Difference	+26.44	+25.26	+0.60	-0.16

The N excreted during the period of decreasing N excretion is almost entirely confined to those catabolites which are of protein origin. The excess of these constitute $\frac{25.26 \times 100}{26.44} =$ 95 per cent. of the total excess loss.

From a quantitative standpoint the protein reserve is small. In this case 26.44 g. in an animal of 130 kg. containing some 3,600 g. protein N. Terroine and Boy found 4.28 g. N as the reserve of a subject weighing 17 kg., and 54 g. in one of 200 kg. If the data derived by this process of reasoning are a correct estimate of the protein reserve of the organism, it is quite obvious that total analysis of the body or of even a tissue will not determine its habitation.

If we adopt Roche's observations as providing a workable hypothesis to account for the retention or loss of N which is observed in the course of the latent period, which elapses before the establishment of a new N equilibrium following a change in the level of the dietary protein or of the total caloric value of a maintenance diet, it does make allowance for the variable

nature of material retained. In this respect it is superior to the theory of a protein reserve analagous in nature to the carbohydrate and fat deposits. The variability of the material retained, at least as judged by its S:N ratio has been worked out by Wilson (1925) who noted that when a basal diet containing no protein is constantly taken, and also during fasting, there is a larger breakdown of S than is accounted for as arising from muscle. On the sudden renewal of the basal diet a preferential retention of S took place indicating that the body may tend to retain its S-containing amino acids which are so essential for oxidation - reduction and other systems. In another interesting experiment Wilson added whites of eggs to his N-poor basal diet and found that two thirds of the extra protein was added to the body and the sulphur retention was such that the retained material had an S:N ratio of 1:15.63, a value comparable to that found for muscle and differing markedly from the S:N ratio obtaining in the egg white. These experiments suggest that the tissues do not simply integrate and disintegrate their proteins in a constant and regular fashion but indicate that the composition of the tissue protein is dependent on the metabolic state at the time.

In the concluding pages of this thesis the writer puts forward a theory to account for the various phenomena observed in the course of this work.

SUMMARY.

1. The superimposition of 160 g. lean topside beef for 5 days, on a diet adequate for maintenance of the adult human organism, resulted in a definite storage of N and S and an increase in body weight. The S:N ratio of the material retained was 1:33.8. The increment in energy value was 200 Cal./day.

2. The addition for 5 days of 46 g. lactose + 35 g. butter to the beef (bringing the protein, carbohydrate and fat up to the levels found in a litre of milk) caused an even greater retention of N and S, and weight increase. The S:N ratio of the material stored was 1:21.6. The additional increment in energy value was 457 Cal. (total increment over basal 657 Cal.)

3. Withdrawal of fat and carbohydrate equivalent to 1430 Cal. led to a rapid fall in body weight and a rise in N and S excretion. The accompanying diuresis was not marked and could account for but part of the rapid fall in weight. By the second day of the low calorie intake (2627 Cal.) slight negative N and S balances were in existence. The rate of N and S loss then remained relatively steady. The S:N ratio of the material stopped being ~~excreted~~ retained was 1:9.00.

The subject of this experiment had previously exhibited a similar rapid decline in body weight without obvious diuresis (extra milk experiment).

The actual increase in weight was 1.03 kg. during the period of surfeit, the calculated weight increase from the N retention was 0.83 kg.

4. On a diet adequate for maintenance carbohydrate equivalent to 780 Cal. was superimposed for 3 days and then removed. A definite retention of N and S and increment in body weight took place. Superimposition of butter equivalent to 700 Cal. effected a less striking N retention. The ratio $\frac{\text{N saved by carbohydrate}}{\text{N saved by fat}}$ per 100 Cal. addition was 2.81.

5. A similar type of experiment on another subject carried out in the reverse order revealed that the addition of glucose, equivalent to 780 Cal., to a diet of 2890 Cal. brought about reductions in the daily urinary N and S of 2.18 g. and 0.156 g. respectively. The S:N ratio of the material retained over 5 days was 1:14.2. Corresponding to this retention of N and S there was a definite increment in body weight.

6. Doubling this intake of glucose caused ultimately a total reduction in the daily N and S outputs of 3.72 g. and 0.287 g. respectively, thus saving 34 and 36 per cent. of the previously metabolised N and S. The S:N ratio of the material retained during this period was 1:13.0. There was a further increment in body weight corresponding to the increased retention. On returning to basal diet a very rapid fall in body weight took place. The slight diuresis which occurred could account for but part of this rapid change in weight.

7. The superimposition of 700 Cal. butter for 6 days also effected a definite though less marked N and S saving effect. On withdrawing the fat basal conditions were only reached after

a period of 5-6 days, indicating that in this respect fat is slow to act but that the action lasts for a considerable time after the fat has apparently ceased to exert its influence. The S:N ratio of the material retained during this period was 1:14.0.

8. The ratio $\frac{\text{N/or S-saving effect of carbohydrate}}{\text{N/or S-saving effect of fat}}$ per 100 Cal. additional intake was for this subject 1.48 for N and 1.69 for S. Under the experimental conditions adopted in these experiments carbohydrate had a greater N and S saving effect than fat.

9. The nature and site of the retained material is discussed.

10. It is concluded that when the energy value of the food intake is in excess of the energy requirements, retention of N and S take place in the form of protein and that fat storage presumably also occurs. Carbohydrate is preferentially burnt.

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PART 4.

OBSERVATIONS ON THE METABOLIC RESPONSE TO INJURY WITH PARTICULAR REFERENCE TO THE DIETARY REQUIREMENTS OF FRACTURE CASES.

So far, this study of the effect of overfeeding on the metabolism of protein has been confined to observations on the normal human subject. In the pages to follow there will be described a series of experiments, which were primarily designed to determine if it was possible, by dietary means, to stem the loss of body substance which is the normal consequence of a severe injury. As has already been mentioned these observations on the injured subject preceded the foregoing experiments in point of time. Had it been possible to reverse the order, the experience gained by the study of the normal would have altered somewhat the procedure adopted in the investigation of the injured subject. Owing to special circumstances a repetition of the earlier work was not possible. However experiments on the rat have helped to clear up certain points which were at issue. These will be described at the conclusion of the present section.

The present writer has drawn attention to the marked loss of body nitrogen, sulphur and phosphorus which occurs in the urine of otherwise healthy individuals who are in receipt of moderate or serious traumatic injury (Cuthbertson, 1930, 1931, 1932, 1934). This loss was observed to commence within a day or two following the injury, to reach a maximum within ten days, and then slowly to decline. It was noted that there generally occurred parallel increments in the basal consumption of oxygen, body temperature and pulse rate. It was suggested that this loss was probably more general than local as in one fracture case 137 g. N, equivalent to about 4.38 kg. of muscle, were lost in

the course of ten days. These phenomena were noted to occur in varying degree in uncomplicated fractures of the long bones, dislocations, effusions into joints, lacerations of soft tissues and surgical incisions into knee joints. In general, the more severe the injury the greater the catabolism. Other experiments indicated that the wasting of muscle and bone caused by immobilisation, which of necessity occurs during treatment of fracture and knee joint cases, was insufficient to account for the whole of this loss of substance though it was undoubtedly a contributing factor. Although there was in most cases an evident initial depression of metabolism following the injury, the counter swing which followed was in excess of an immediate restitution.

As the accompanying fever was generally slight or scarcely appreciable in the milder cases, and as a study of pneumonia cases before and after the crisis had failed to reveal catabolic effects in excess of those noted in severe injuries to the limbs, in particular those due to direct violence, it was considered that the loss of body substance was not proportional to the fever.

I. Note on the effect of injury on the level of the plasma proteins.

Yet another phase of this disturbance of metabolism caused by injury has come to light, namely, the changes induced in the level of the plasma proteins. In the pursuit of this particular investigation I owe much to the help of Dr. S. L. Tompsett, until lately my assistant.

It has long been known that the level of fibrinogen in the blood plasma is very variable, and that acute inflammations are invariably associated with high values. Further, a deficit of fibrinogen in contrast to the other proteins can be restored

in a few hours. Foster and Whipple (1921-22) showed that dead tissue and a non-specific (aseptic) inflammation are the only factors necessary to cause this increase of fibrinogen in the blood. W. and H. Lohr (1922) and Govaerts and de Harven (1928) noted a rise in the fibrinogen content of the plasma following aseptic operations. In addition W. and H. Lohr observed a reversal in the albumin: globulin ratio following aseptic operations and accidental injuries. The absolute amounts of albumin and globulin, however, are not recorded in their paper. Reinmann, Medes and Fisher (1933) believe that the increases in globulin and fibrinogen may, in part, be due to the decomposition of leucocytes, especially of the polymorphonuclear type in the circulation or in the haematopoietic system.

The following observations were made with a view to assessing the time and nature of the changes in the level of the plasma proteins which result from traumatic injury and surgical intervention.

EXPERIMENTAL.

Methods Employed.

The venous blood was withdrawn without stasis into tubes containing a standard minimum amount of oxalate. Peters, Eisenmann and Bulger (1925) have found that oxalate causes a dilution of the plasma such as to reduce the total protein content by 0.3-0.4 g. per 100 ml., and recommended the use of heparin. Although heparin was not used in the present series of observations, the dilution effects would be relatively constant and

should not affect the significance of the data, since the emphasis is upon the relative and not the absolute changes in the level of the plasma proteins.

The fractionation and determination of the plasma proteins was performed by the composite method described by Hawk and Bergeim (1927).

Results.

Salvesen's (1926) data on sixteen normal men may be taken as a basis from which to judge the deviation found to result from injury. The maximum and minimum values found for albumin were 5.24 g. and 3.95 g. per 100 ml. plasma (mean 4.44); for globulin, including fibrinogen, 3.18 g. and 1.96 g. (mean 2.58). Peters and Van Slyke (1931) quote as extreme values for fibrinogen 0.4 g.-0.2 g. Rowe (1917) has noted that excessive protein feeding does not increase the plasma protein content above the usual normal limit, so that it is unlikely that the effects noted in Cases 1 and 3 were due to the large amounts of egg-white and sodium caseinate which they respectively consumed. Schenk and Kunstmann (1933) have found that the level and amino-acid constitution of the proteins of the blood serum may vary from individual to individual, and in the same individual during the course of the day. The main differences occurred in the albumin fraction.

The details of the cases investigated are given in the table.

Case 1 exhibits the series of changes in the plasma proteins following an injury such as the fracture of both bones

Case.	Time after accident or operation.	Plasma protein N g. Total.	Albumin.	Globulin.	Fibrinogen.	Plasma N.P.N. mg.	Nature of accident or operation.
1	5 hours	7·11	4·14	2·80	0·17	24	Fracture both bones of both legs by direct violence. Legs manipulated under general anæsthetic 9–10 hours after accident.
	24 „	8·05	4·54	3·15	0·36	30	
	2 days	9·45	3·18	5·98	0·29	30	
	3 „	9·30	3·22	5·78	0·30	25	
	4 „	7·71	3·54	3·97	0·20	26	
	6 „	9·49	2·50	6·61	0·38	32	
	7 „	9·49	3·13	6·36		25	
	8 „	7·97	3·24	4·50	0·23	24	
	16 „	7·78	4·86	2·74	0·18	26	
2	22 hours	6·80	3·02	3·78		23	Fracture of femur.
	2 days	8·56	2·90	5·40	0·26	26	
	4 „	6·80	3·21	3·36	0·23	27	
	6 „	8·41	2·96	5·24	0·21	26	
	9 „	7·55	3·50	3·86	0·19	24	
	11 „	7·24	3·34	4·64	0·26	26	
	14 „	7·55	3·46	3·92	0·17	26	
3	24 hours	6·98	2·64	3·71	0·63	23	Compound fracture of both bones one leg. On 17th day manipulated under general anæsthetic and 2½ hours later blood withdrawn.
	7 days	9·71	2·95	6·30	0·46	32	
	14 „	6·96	3·58	3·15	0·23	32	
	17 „	15·06	4·92	9·07	1·07	43	
	18 „	10·97	5·38	5·00	0·59	33	
	21 „	7·10	3·15	3·78	0·17	20	
4	Before operation	9·56	4·10	5·46		31	Excision of knee-joint under general anæsthetic (35 min.) to correct deformity due to old osteomyelitis in childhood; now 17½ years.
	1½ hours	9·81	4·05	5·40	0·36	20	
	2¾ „	11·42	3·79	6·82	0·81	23	
	5¼ „	11·73	3·79	7·08	0·86	24	
	24 „	13·01	2·63	9·64	0·74	24	
	2 days	10·21	3·91	6·10	0·20	33	
	3 „	10·08	3·78	6·14	0·16	30	
	6 „	10·08	3·78	6·05	0·25	26	
	14 „	6·89	4·56	2·14	0·19	26	
	22 „	6·28	3·78	2·34	0·16	26	
5	1 day	6·80	3·02	3·78		23	Effusion into knee-joint as result of fall from car. This case was of same sex, build and age as Case 6.
	2 days	8·56	2·90	5·40	0·26	26	
	4 „	6·80	3·21	3·36	0·23	27	
	6 „	8·41	2·96	5·24	0·21	26	
	9 „	7·55	3·50	3·86	0·19	24	
6	2 days	6·86	2·80	4·06		26	Effusion into knee-joint as result of fall.
	3 „	12·15	2·71	9·12	0·32	24	
	5 „	7·23	4·21	2·83	0·19	26	
	7 „	8·80	3·78	4·79	0·23	24	

of both legs. The first observation made 5 hours after the accident and before manipulation under anaesthetic, revealed a normal partition of the proteins. Subsequent analyses showed a rise in total protein due to marked increase in the globulin fraction accompanied by a slight rise in fibrinogen. The albumin moiety declined. These changes subsided about the fourth day, and then returned to their previous level about the end of the first week. At the end of 16 days a normal ratio was found. Irregular fluctuations, chiefly in the globulin fraction, were also noted in Case 2. That these fluctuations in the level of the plasma proteins may follow disturbances at the injured site is noted in Case 3. This case also exhibited a rise, then a fall in the globulin fraction. On the seventeenth day after the accident the leg was manipulated under anaesthesia (chloroform and ether). A very marked rise in the total protein was noted in a sample of blood withdrawn $2\frac{1}{2}$ hours after this procedure. The rise was principally due to a great increase in globulin and fibrinogen fractions. This change soon subsided.

The most complete record of these fluctuations following injury is that of Case 4. It includes data collected both before and after an excision of the knee-joint of a lad of $17\frac{1}{2}$ years. This operation was performed to correct a deformity due to osteomyelitis in childhood. The pre-operation level of the total protein was high, due to an abnormal content of globulin, possibly caused by the persistence of some chronic inflammatory process. As a result of the operation there was a rapid and

and marked rise in globulin and fibrinogen accompanied by a slight rise in the albumin fraction. The maximum change was noted 24 hours after the operation. Subsequently both globulin and fibrinogen declined, while the albumin fraction rose. Haematocrit determinations did not indicate any change in blood volume to account for these marked fluctuations.

That anaesthesia and actual fracture of bone may be discounted as agents peculiar to this disturbance in the plasma proteins may be inferred from Cases 5 and 6. Each of these men, who were of similar build and age, suffered from an effusion into a knee-joint as the result of a fall. It will be noted that also in these cases there was a rise in the plasma proteins mainly due to increases in the globulin fraction. The maximum recorded changes varied in time and degree. Fuller investigation of the first 24-hour period might have revealed earlier changes.

DISCUSSION.

These observations have shown that trauma such as to cause the fracture of one or more of the long bones, or an effusion into a knee-joint or a surgical procedure such as the excision of a knee-joint, causes an immediate and marked disturbance in the total amount and relative proportions of the different plasma proteins as fractioned by neutral salt precipitation. The normal level is slowly regained.

The general effect is a slight fall in the albumin moiety coupled with a marked rise in the globulin fraction.

Fibrinogen is often appreciably raised.

A satisfactory explanation of these phenomena cannot be offered, although somewhat similar changes have been observed in the level of the blood fat, following operation (Prochnow and Findeisen, 1933). In surgical shock and allied states the plasma proteins do not rise, but fall, in spite of the fact that the haemoglobin increases (White and Erlanger, 1920). In the present series of cases shock was practically absent, and the injury was rapidly succeeded by a period characterized, in particular, by an increase in globulin and fibrinogen. This change is probably intimately related to the process of healing, involving as it does proliferation and phagocytosis. Hyperaemia in the healing area probably results in increased permeability of the capillary walls to plasma colloids, in the same way that occurs in acute inflammation. Ostwald (1910) has shown that the proteins of the plasma are arranged in an order of permeability according to the following series: albumin $>$ globulin $>$ fibrinogen. The fall in the level of albumin might thus be accounted for. It is more difficult to account for the rise in globulin and fibrinogen unless these be regarded as compensatory increases to maintain the osmotic pressure exerted by the plasma colloids.

Whipple and his co-workers (1935) state that in an emergency plasma protein may contribute to body protein. They found that in their dogs there was a "reserve store" of plasma-protein-building substance which might amount to 30-120 g. of potential material - twice or three times as much on the average as the dog has in its circulation. They suspect the liver and

perhaps other tissues as the reserve store for this material, which may be used for plasma protein regeneration or for tissue repair. This is in general agreement with the views of Reinmann, Medes and Fisher (1934). That the plasma proteins can be utilized in the body economy appears evident from the experiments of Holman, Mahoney and Whipple (1934), which demonstrate that dogs receiving only sugar by mouth can be maintained practically in N equilibrium by suitable amounts of blood plasma given intravenously.

The recent work of McFarlane (1935) has thrown doubt on the homogeneity of the globulin fraction as partitioned by neutral salt precipitation. A variety of sera - normal and pathological, human, cow and horse - were treated with ammonium sulphate in accordance with the usual fractionation technique. The globulin precipitates were redissolved in 1 p.c. NaCl, dialysed free of SO_4 ions and examined in the ultracentrifuge. In some cases practically no albumin was present; in others there were two sedimenting boundaries corresponding in sedimentation rate to albumin and globulin - indeed as much as 40% of this fraction was sometimes albumin. There appears to be no simple explanation of this phenomenon.

So far no attempt has been made to apply ultracentrifugal analysis to the plasma of injured persons, so that it cannot be stated that the observed increase in globulin is a true increase. Should it be found that the observed increase is due to the presence of albumin, the marked increase in albumin still demands an explanation.

It is of interest to note that although the maximal changes in the plasma proteins precede in time the phase of increased catabolism which follows injury, and which reaches a maximum within 4-10 days from the date of injury (Cuthbertson, 1930), it is probable that these phenomena are intimately related to the processes of repair and maintenance.

II. Observations on the dietary requirements of fracture cases.

Although it is hardly to be expected that the innate reparative processes could be influenced by dietary means this cannot be assumed with certainty until proof to the contrary is forthcoming.

Clark (1919) noted that a high protein diet tended to eliminate the latent period in the healing of wounds though the periods of contraction and epithelium formation were unchanged. A high fat diet prolonged the latent period for some six days, whereas a high carbohydrate diet was intermediate in effect. Several workers have confirmed the stimulating effect of protein on cellular proliferation (Smith and Moise, 1924; Carrel and Baker, 1926; Osborne and Mendel, 1926). The clinical observations of Herrmannsdorfer (1927) which have been confirmed by Reimers and Winkler (1933) on the dog with NH_4Cl, appear to indicate that an acid diet facilitates the healing process, particularly in the way of restraining the bacterial flora.

There are indications that in aseptic wounds there is a rise in the hydrogen ion concentration of the tissue fluids of the traumatized area. By use of the subcutaneous electrode method of gas chain measurement Girgolaff (1924) found a shift of pH from approximately the normal value of 7.2 to 6.32 in 24 hours.

The influence of diet on the regeneration of tissues can only be of very secondary importance for, as Morgan (1906) long ago demonstrated, salamanders regenerate their amputated legs in the absence of food just as rapidly as do well-fed animals. Howes, Briggs, Shea and Harvey (1933) found too that the rate of healing of stomach wounds of adult rats was not noticeably affected by complete starvation or by a half-adequate diet. The healing rate in young rats did appear to be affected.

Experimental.

The patients were supplied with the requisite foodstuffs prepared in an appetising form. It was generally found that after about a fortnight of high protein diet their appetite failed, and though the patients were still willing to co-operate they could not ingest the required amount.

Difficulty was experienced in deciding the period of time over which the net gain or loss of body substance should be assessed. It was finally decided to omit the first three days of diet from the final computation.

The experiments are arranged in groups and although not strictly chronological, this order is the more convenient.

The analytical methods used were in general, similar to those adopted in previous experiments.

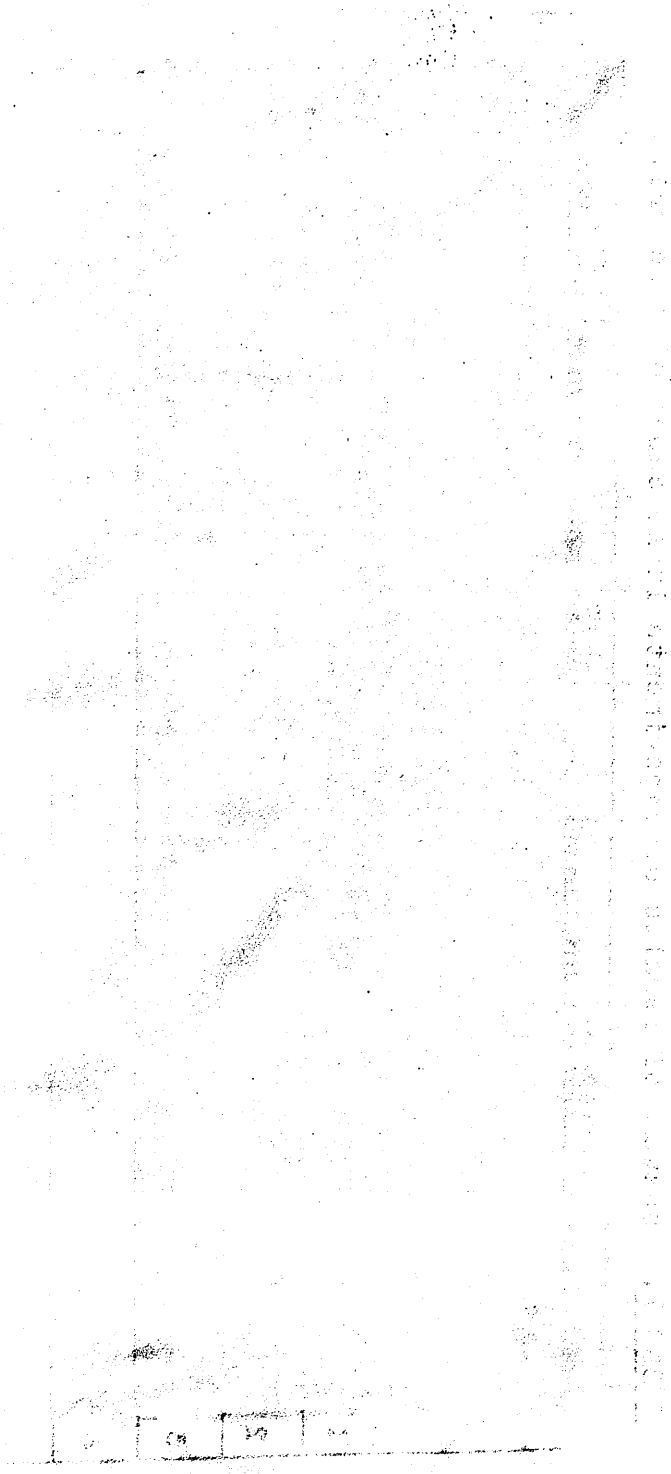


TABLE 1. Summary of results of experiments in 16 cases of fracture.

Case	Age	Period of Observation Reckoned from Day of Accident	Nature of Injury	Total Calories	N Intake (Grm..)			Maximum Urinary Excretion of N (Grm.)	Day of Maximum Urinary N Excretion Reckoned from the Time of Accident	N Excretion (Grm.) Excluding First Three Days of Observation			N Balance Excluding First Three Days of Observation (No. of Days)
					Basal N	Additional N and Source	Total N for Period (Average Daily)			Urine	Fæces (Average Daily)	Total N for Period	
1	43	2–14	Fractured humerus	1981	128·40	—	128·40 (14·27)	21·87	10th	154·90	11·52 (1·28)	166·42	— 38·02 (9)
2	59	3–14	Fractured humerus	1944	127·40	—	127·40 (14·16)	15·89	9th	135·01	8·64 (0·96)	143·65	— 16·25 (9)
3	24	2–17	Fractured tibia	1802	172·21	45·23 (Bovril)	217·44 (16·84)	36·18	7th	375·34	21·58 (1·66)	396·92	— 179·48 (13)
4	17	2–14	Lacerated tendo Achillis	2200	153·80	14·91 (Glycine)	168·71 (16·87)	19·31	6th	171·61	11·80 (1·18)	183·41	— 14·70 (10)
5	21	2–22	Almost complete fracture of both bones of one leg	1990	196·20	47·88 (Eatan)	244·08 (13·56)	21·56	11th	338·65	17·46 (0·97)	356·11	— 112·03 (18)
6	20	2–14	Fractured tibia	3259	119·50	199·74 (Gelatin)	318·24 (31·82)	42·36	5th	370·36	12·48 (1·25)	382·84	— 64·60 (10)

TABLE 1. (Contd.) Summary of results of experiments in 16 cases of fracture.

Case	Age	Period of Observation Reckoned from Day of Accident	Nature of Injury	Total Calories	N Intake (Grm.)			Maximum Urinary Excretion of N (Grm.)	Day of Maximum Urinary N Excretion Reckoned from the Time of Accident	N Excretion (Grm.) Excluding First Three Days of Observation			N Balance Excluding First Three Days of Observation (No. of Days)
					Basal N	Additional N and Source	Total N for Period (Average Daily)			Urine	Fæces (Average Daily)	Total N for Period	
7	25	2–16	Fractured tibia and fibula of one leg	3097	117·84	347·40 (Gelatin)	465·24 (38·77)	43·31	7th	464·10	13·08 (1·09)	477·18	11·94 — (12)
8	23	2–27	Fractured humerus with bruising and laceration over supra-orbital ridge	2386	217·92	531·36 (Sodium caseinate)	749·28 (32·57)	38·51	6th	726·53	36·50 (1·59)	763·03	— 13·75 (23)
		5–14		2386	99·88	243·54 (Sodium caseinate)	343·42			365·38	17·63	383·01	— 39·59 (10)
		15–27		2386	118·04	287·82 (Sodium caseinate)	405·86			361·15	18·87	380·02	+ 25·84 (13)
9	26	2–12	Compound fracture of tibia and fibula of one leg	5392	79·55	227·70 (Sodium) caseinate)	307·25 (34·14)	42·39	11th	342·29	15·84 (1·76)	358·13	— 50·88 (9)
10	42	2–11	Fractured tibia and fibula at lower end of one leg	3557	166·74	—	166·74 (23·82)	30·69	6th	197·62	5·91 (0·84)	203·53	— 36·79 (7)
11	35	2–17	Compound fracture of tibia and fibula of one leg	3193	345·41	—	345·41 (26·57)	31·59	7th	354·99	17·68 (1·36)	372·67	— 27·26 (13)
12	38	3–15	Fractured tibia and fibula of both legs	3214	318·40	—	318·40 (31·84)	40·25	8th	318·89	17·10 (1·71)	335·99	— 17·59 (10)
13	23	1–18	Fractured tibia and fibula of one leg	3044	507·60	—	507·60 (33·84)	42·00	6th	523·72	29·70 (1·98)	553·42	— 45·82 (15)
14	44	2–21	Fractured tibia and fibula of one leg	3606	623·73	—	623·73 (36·69)	40·82	8th	609·78	39·44 (2·32)	649·22	— 25·49 (17)
15	31	2–13	Fractured fibula	4135	147·51	—	147·51 (16·39)	22·96	6th	172·05	11·36 (1·26)	183·41	— 35·90 (9)
16	16	1–8	Fractured tibia and fibula of one leg	3592	41·55	—	41·55 (8·31)	20·67	4th	80·12	9·00 (1·80)	89·12	— 47·57 (5)

Urinary K was determined by the method of Kramer and Tisdall (1921) with slight modification. After separation of the potassium cobalti-nitrite it was oxidised with a known excess of standard permanganate as in the original method, but the excess after oxidation was determined by the addition of Kl (Kahlbaum) and titration of the liberated I_2 with $\frac{N}{200}$ sodium thiosulphate.

Sodium in the urine was determined by the method of Kramer and Gittleman (1924) following the incineration of 50 ml. urine acidified with H_2SO_4 at as low a temperature as possible. The residue was dissolved in water and made up to 50 ml. 1 ml. was then taken for analysis.

Before proceeding to describe the experiments on forced feeding it is of interest to record the N balance in two cases in which disuse atrophy was reduced to a minimum.

The effect of gentle massage and movements in the treatment of two ambulant cases with fractured humeri.

Cases 1 and 2 were placed on diets of average first class protein content (14.27 g. and 14.16 g. N per day respectively). In the course of 9 days Case 1 lost 38.0 g. N and Case 2 lost 16.25g. N. (The metabolic data are given in Table 1.)

A kick from a horse fractured the humerus of Case 1 at the junction of lower and middle thirds. Case 2 was injured at practically the same site by a fall of stone. There was also some bruising and laceration at the site of injury in the latter case.

The daily fluctuations in the urinary volume bore a fairly close relationship to the total N excretion.

In these two cases disuse atrophy was reduced to a minimum by allowing the patients freedom to walk about hospital, and by massaging and manipulating the injured area daily from the date of injury. It is evident that this procedure did not eliminate the loss of body N. These two cases were the oldest of the series being 43 and 59 years respectively. This disparity may have been a factor in deciding the degree of catabolism.

The effect of meat extractives. Case 3, Fig.1.

Case 3, who was on a moderately low first class protein diet, received in addition 60 g. meat extractive (Bovril) daily, equivalent to an extra 3.48 g. N.

In the course of 13 days Case 3 (fractured tibia) lost 179.4 g. N (equivalent to about a stone of muscle if that be the source of this N). On the 13th day following the accident Case 3 had an infarction of lung and there was an abrupt rise of body temperature coupled with a slight rise in the urinary volume and N excretion. These rises subsided after three days but the temperature subsequently rose and clinical evidence of pneumonic consolidation was present. Unfortunately this development concluded the biochemical record. It was apparent that even with definite fever the rise in N excretion was not as great as occurred in consequence of the initial injury.

Throughout the period of observation this case was on a constant intake of water, the volume of urine varying with the N excretion.

Fig. 1 Case 3.

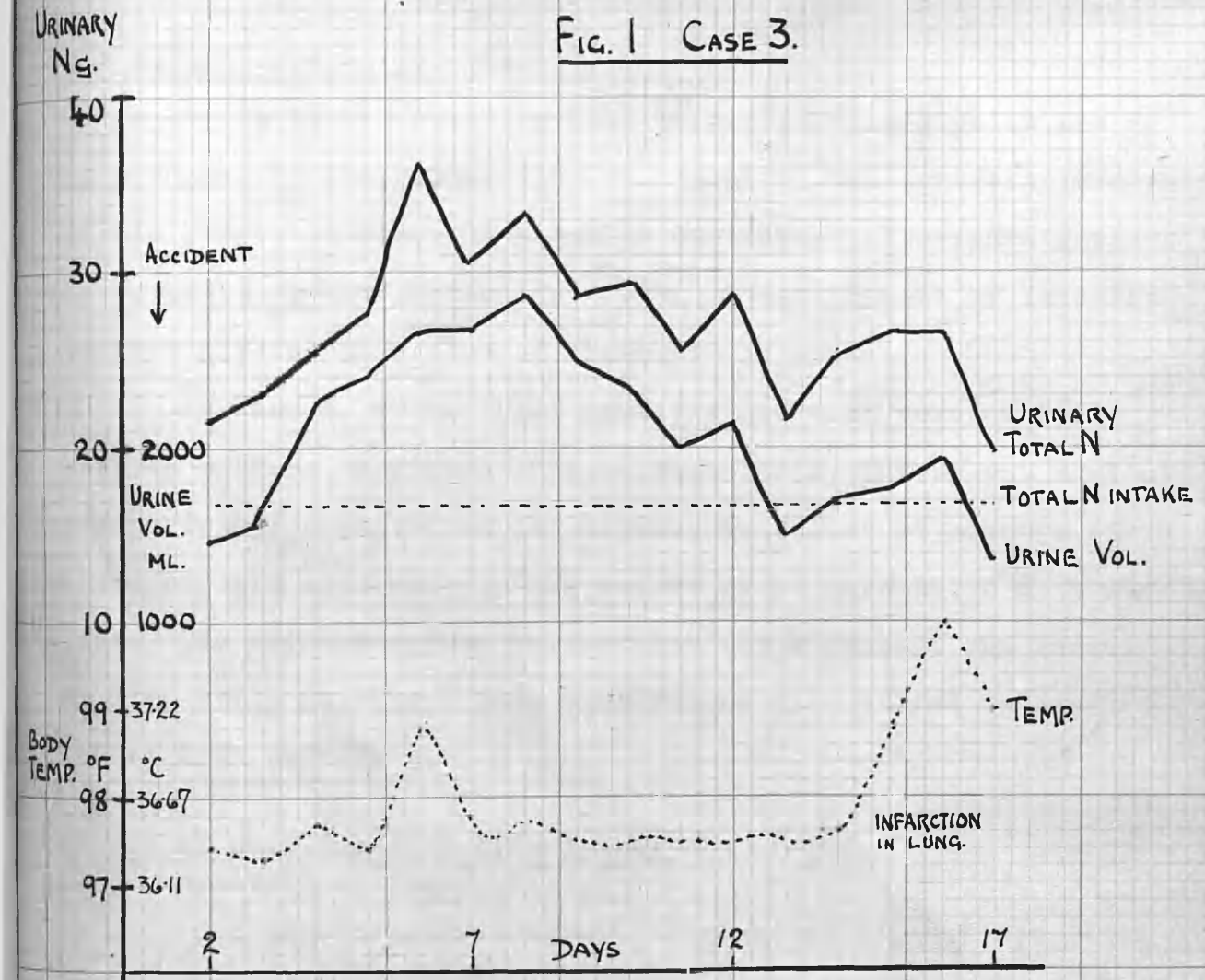
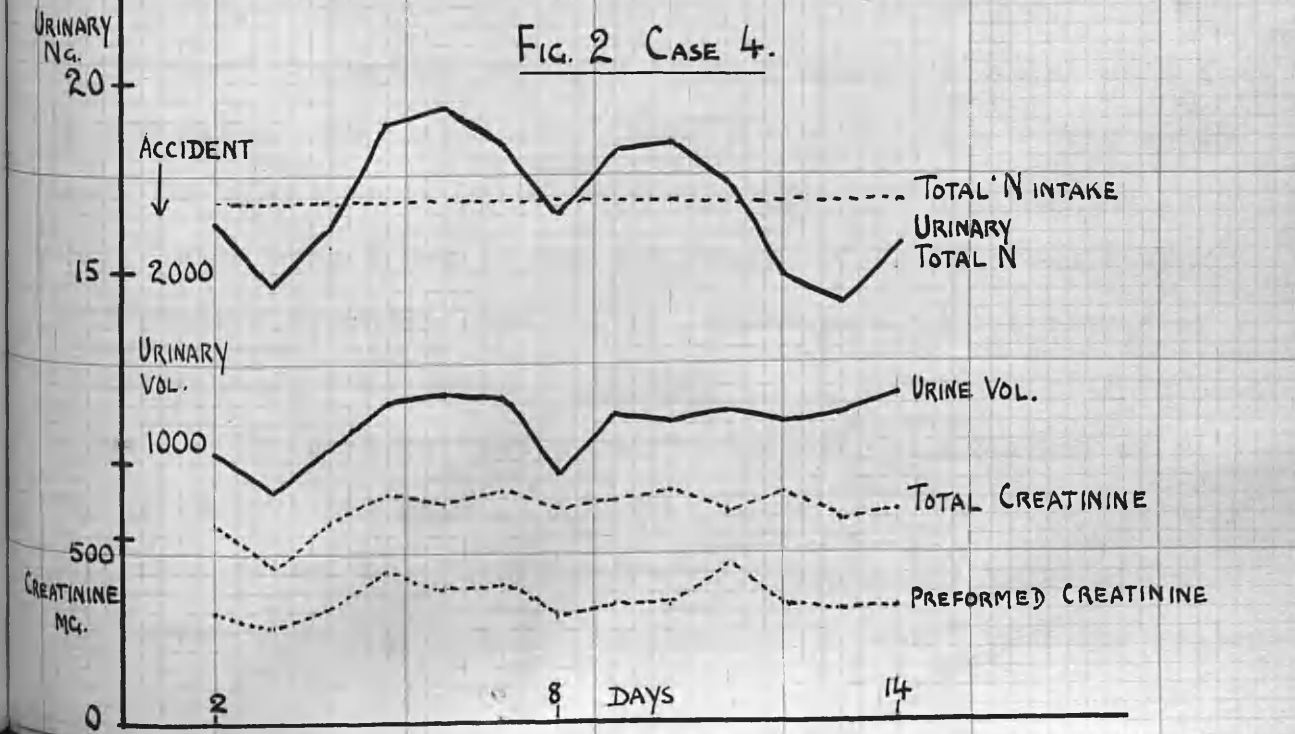


Fig. 2 Case 4.



The effect of glycine. Case 4, Fig. 2.

As it has recently been suggested that glycine may play a part in the restoration to normal of the partially atrophied muscle fibres in cases of muscular dystrophy (literature reviewed by Cuthbertson and Maclachlan, 1934), it was thought of interest to feed glycine to a case of traumatic injury.

Case 4, whose tendo Achilles had been severed by a reaping machine, received 10 g. glycine daily throughout most of the period of observation, in addition to a diet of average first class protein content. In the course of 10 days 14.70 g. N were lost. The urinary volume varied with its N content but tended to rise still further. Body temperature also varied to some extent with urinary N.

Effect of addition of a hydrolysate of mixed ox tissue. Case 5, Fig. 3.

A palatable hydrolysate of mixed ox tissues was found in the preparation Eatan. About 71% of the total N of this proprietary product is reported to be combined in amino acid form. To the rather low first class protein diet of Case 5 were added daily 50 ml. (2.53 g. N) of this mixture. The patient - a footballer with both bones of one leg fractured as the result of kicking an opponent's boot - lost 112.03 g. N in the course of 18 days.

The urinary volume fluctuated with its content of N. During the gradual rise in N output there were periodic rises in body temperature, but these had passed off and the temperature had become normal before the maximum daily output of N was reached.

Fig 3 Case 5.

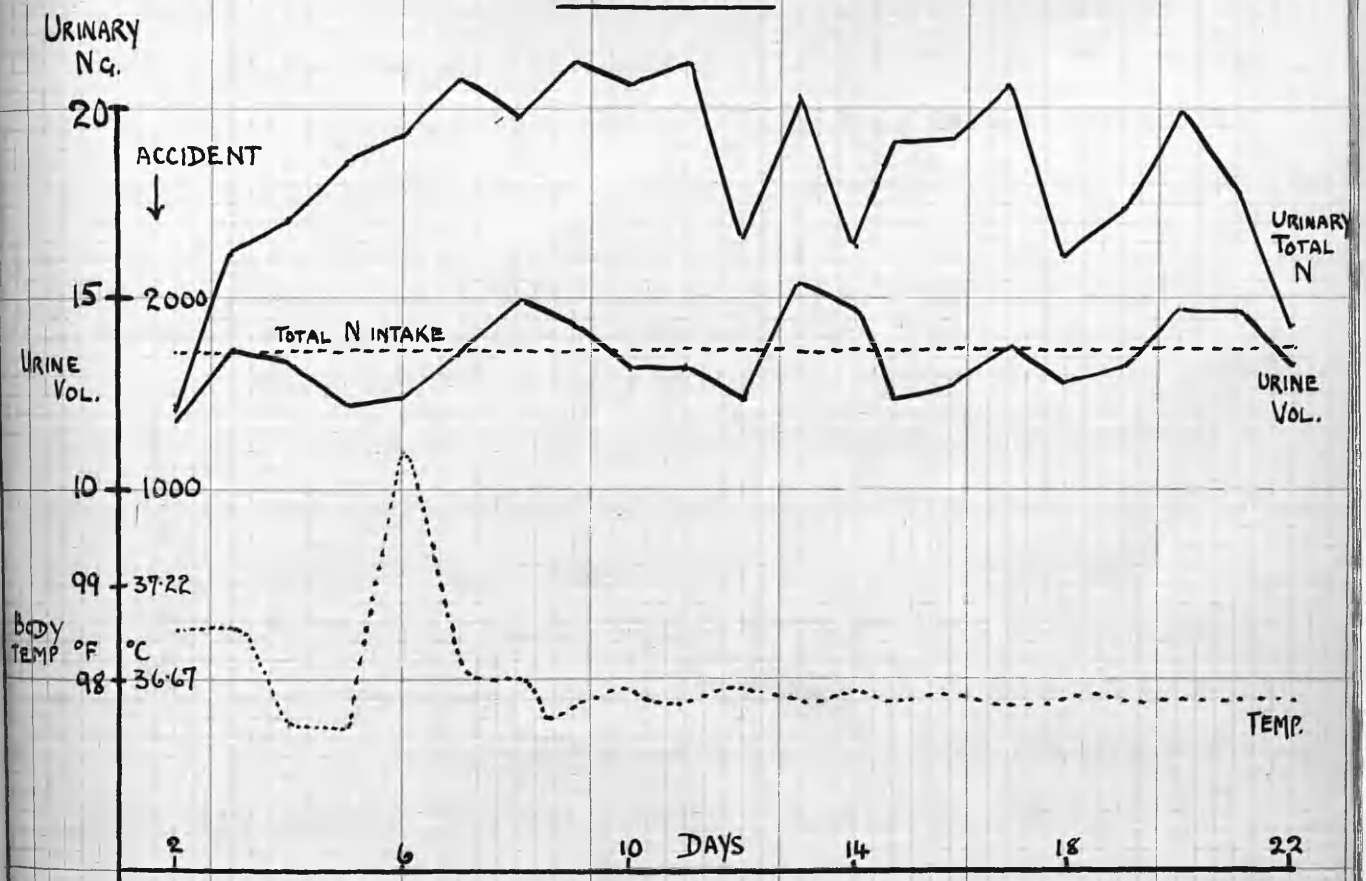
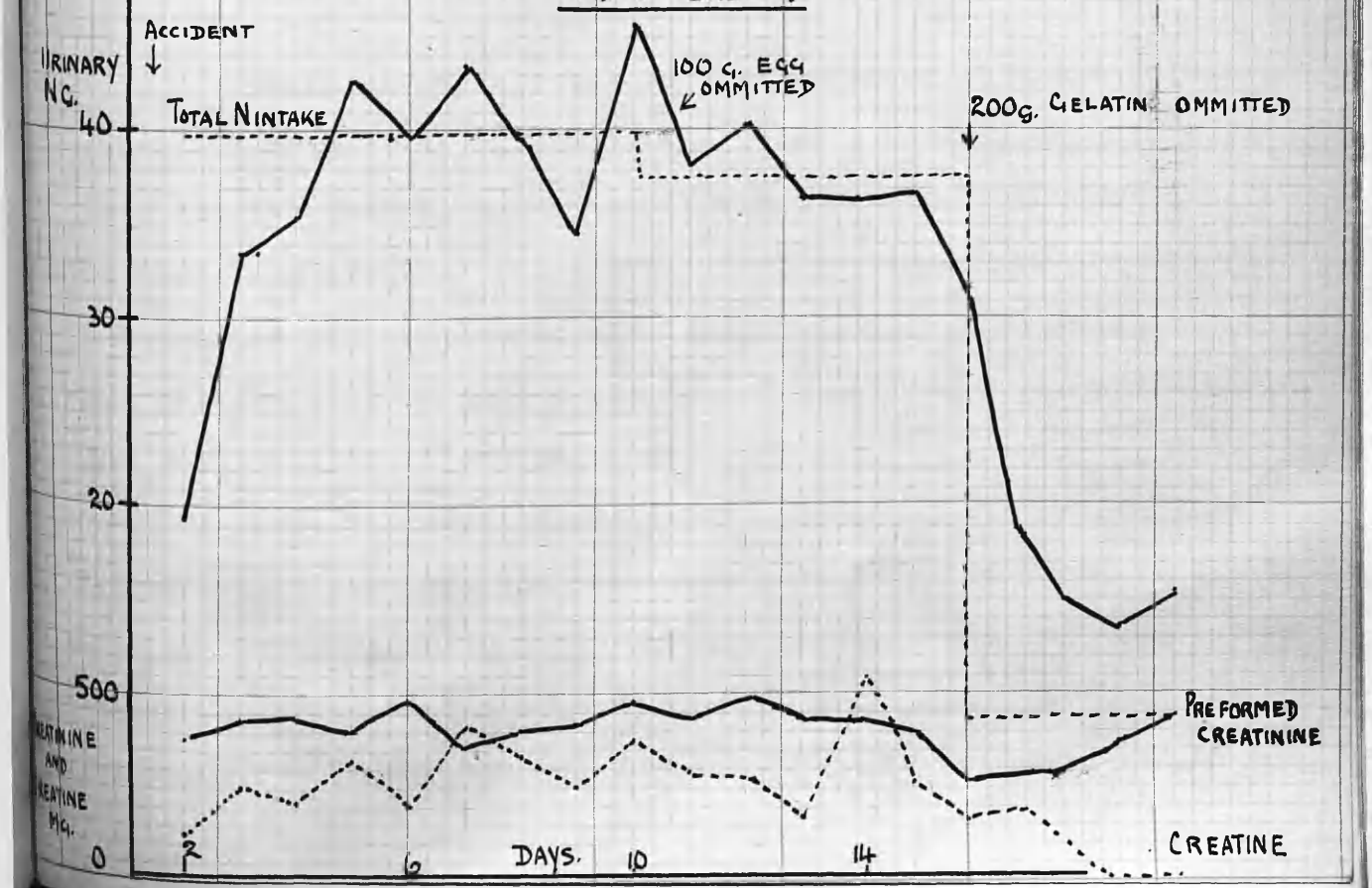


Fig. 4 Case 7



It was thought that some measure of success in stemming the loss of body N might be achieved by adding certain proteins in large amount to the diets of injured persons.

Effect of addition of gelatin (up to 200 g. daily) to diets of low first class protein content.

Case 6 fractured his right tibia as the result of indirect violence there being no apparent displacement of the bone ends. From the third to seventh days he received daily 120 g. gelatin and then 150 g. daily for six days. The basal diet was low in protein (mainly second class) and more than adequate in energy value (3259 Cal.). The patient lost 64.60 g. N in 10 days.

Urinary N and volume ran parallel. When the gelatin was finally omitted from the dietary the urinary volume did not decline proportionally. The body temperature changes paralleled the N excretion.

Preformed creatinine initially varied with the urinary volume and urinary N excretion, but later did not fluctuate with these changes tending to remain fairly constant. The creatinuria was almost as great as the excretion of preformed creatinine. Increment in gelatin intake led to an increase in creatine excretion and decrease in intake led to a diminution in the creatinuria. This point will be discussed later.

Case 7, Fig.4, whose injury consisted of the fracture of both bones of one leg, the result of a kick on the football field, received 200 g. gelatin daily throughout the period of observation the energy value of the diet being 3097 Cal. From the 16th day following the injury the gelatin was omitted. From the second to

sixteenth days the patient lost 15.30 g. N.(from 5th-16th, 11.94g.N)

The urinary N varied with the total volume of urine passed during the gelatin feeding period. The body temperature was never above 36.83°C during the whole period of observation.

The preformed creatinine excretion was very constant. The creatinuria was marked and its daily fluctuations corresponded with the changes in total urinary N. On ceasing to give gelatin it quickly declined and within three days only a trace was present.

The most marked creatine excretions of the series were noted in these two cases and in Case 4 who received 10 g. glycine daily. The degree of creatinuria in the former two cases appeared to be definitely related to the ingestion of gelatin and, since glycine itself appeared to produce a similar effect, it may be reasonable to assume that the increased creatinuria induced by gelatin feeding was related to its high glycine content.

Many years before Gibson and Martin (1921) had noted that the administration of gelatin to cases of muscular dystrophy led to a creatinuria. These observations in injured subjects suggest that their muscular function is deranged in some peculiar way. It has long been known that fracture cases frequently exhibit a creatinuria.

<u>The effect of sodium caseinate (175-200 g. "Protosol" daily) when added to diets of very low first class protein content.</u>

Case 8, Fig.5 received his injuries - a fractured humerus with bruising plus a lacerated wound over right supraorbital ridge - as the result of colliding into a wall when bicycling. His arm was massaged daily and gently moved before being put back into

Fig. 5 Case 8

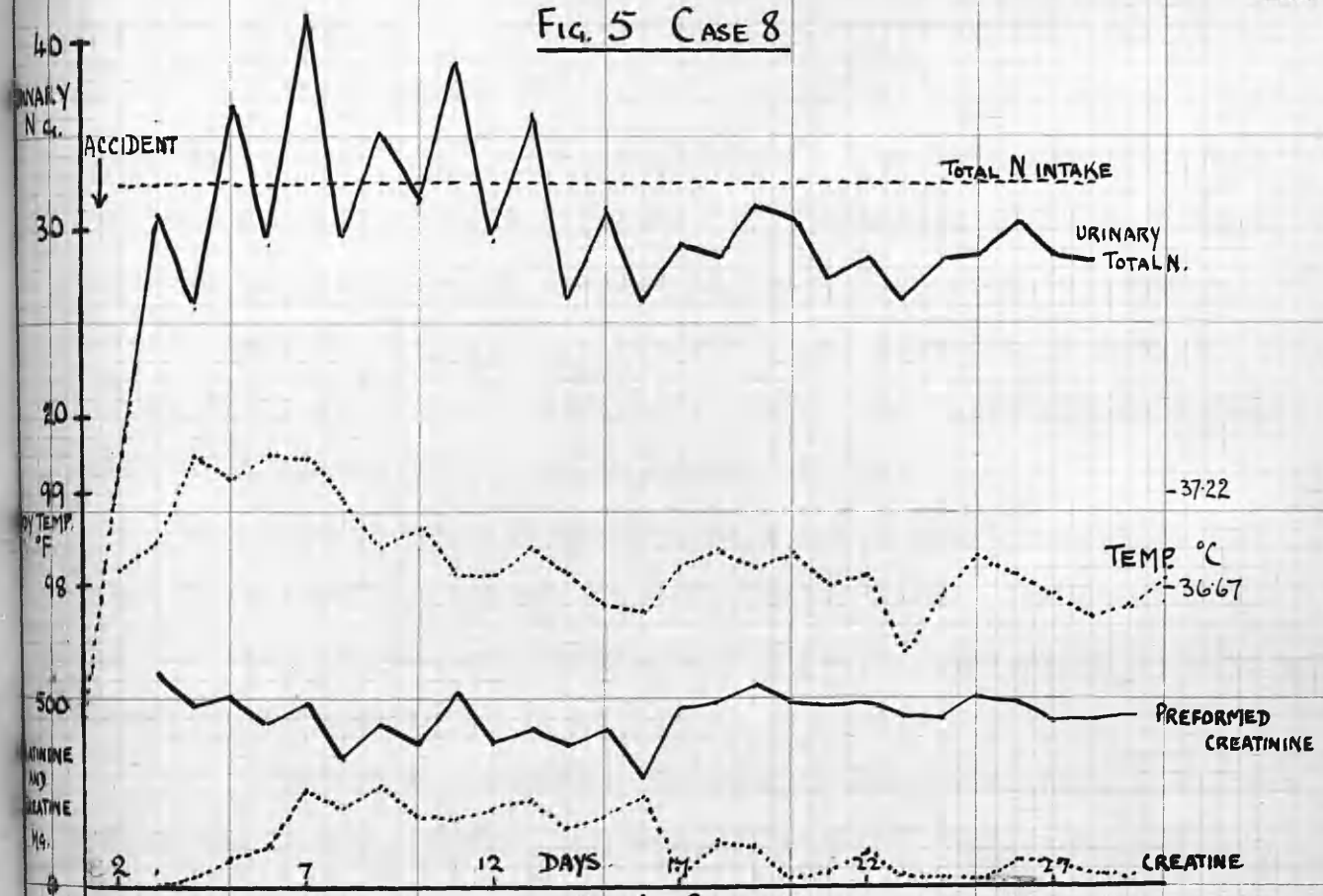
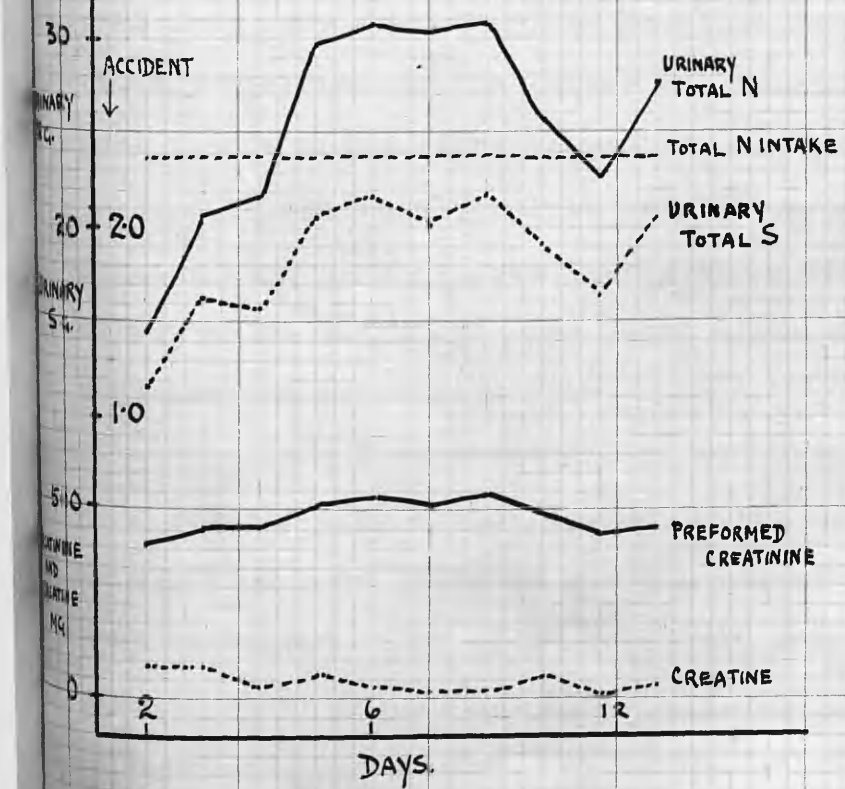


Fig. 6 Case 10



the splint. For the first week he stayed most of the time in bed as his body temperature was slightly above 37°C for some six days. The patient lost 13.75 g. N. in 23 days. The main loss of substance took place during the first ten days when 39.59 g. N were excreted in excess of intake and from the fifteenth to twenty-seventh days 25.84 g. N were retained. In addition to a diet low in first class protein and believed to be just adequate in energy value he consumed 175 g. sodium caseinate daily.

The urinary volume fluctuated with the N excretion but later continued to rise as the N gradually fell. The urinary N and the body temperature curves were parallel, the daily fluctuations corresponding.

Preformed creatinine fluctuated with the urinary changes but as the urinary N rose the preformed creatinine decreased slightly, rising again as the urinary N fell. Creatine excretion rose from zero then remained parallel to the total urinary N and finally declined as the latter fell becoming eventually little more than a trace. The degree of creatinuria is apparently related to the total protein catabolism and this experiment would suggest that as this creatinuria waxes the preformed creatinine output wanes slightly. The basal diets of Cases 8 and 9 were creatine- and creatinine-free.

Case 9 received 200 g. sodium caseinate daily, in addition to a grossly over adequate diet, during his convalescence from the fracture of both bones of one leg caused by direct blow by a machine handle. The wound, which was compound, was incised and cleaned under chloroform and ether anaesthesia. No sepsis resulted.

Despite this degree of over-feeding the patient lost 50.88 g. N during 9 days. The urinary volume rose with increase in N excretion and continued rising even after the N had reached its maximum value. The maximum body temperature (37.44°C) coincided with the maximum N excretion.

Preformed creatinine remained fairly constant though as in the previous case a tendency to fall as the creatinuria developed, was noted. The latter initially varied with the total N excretion but later fell although the N output still remained high.

The effect of diets rich in first class protein and energy value.

Case 10, Fig.6 had the lateral and medial malleoli of the bones of one leg fractured as the result of a waggon passing over his leg just above the ankle. Though the diet contained 23.82 g. N and 3557 Cal. daily, the patient during 7 days of observation lost 36.79 g. N. The total urinary S fluctuated in parallel with the total N and urinary volume. As no precaution had been taken to prevent S loss in the faeces during drying, only the excess outputs of N and S in the urine can be compared. On this basis the S:N ratio of the material lost was 1:16.36. The S:N ratios of intake and total urinary outputs were 1:13.65 and 1:13.79 respectively. The S:N of the urinary excretions during the first and second periods of five days were 1:13.63 and 1:13.94 respectively.

The excretion of preformed creatinine rose slightly, the rise corresponding to the rise in total N and S. The creatinuria diminished slightly. Owing to the fact that the patient was

on a meat containing diet perhaps little value can be attached to this apparent reciprocal relationship. It is of interest to note it in passing. In this case there was practically no disturbance of body temperature.

Case 11, Fig.7 received a compound fracture of both bones of one leg (at their mid point) as the result of having his leg crushed between two large drain pipes.

During the period of observation (13 days) he lost 27.26 g. N, the daily intake being 26.57 g. and energy value of the diet 3193 Cal. The excretion of total sulphur in the urine varied with that of total nitrogen, and the volume of urine passed showed similar changes though occurring somewhat later.

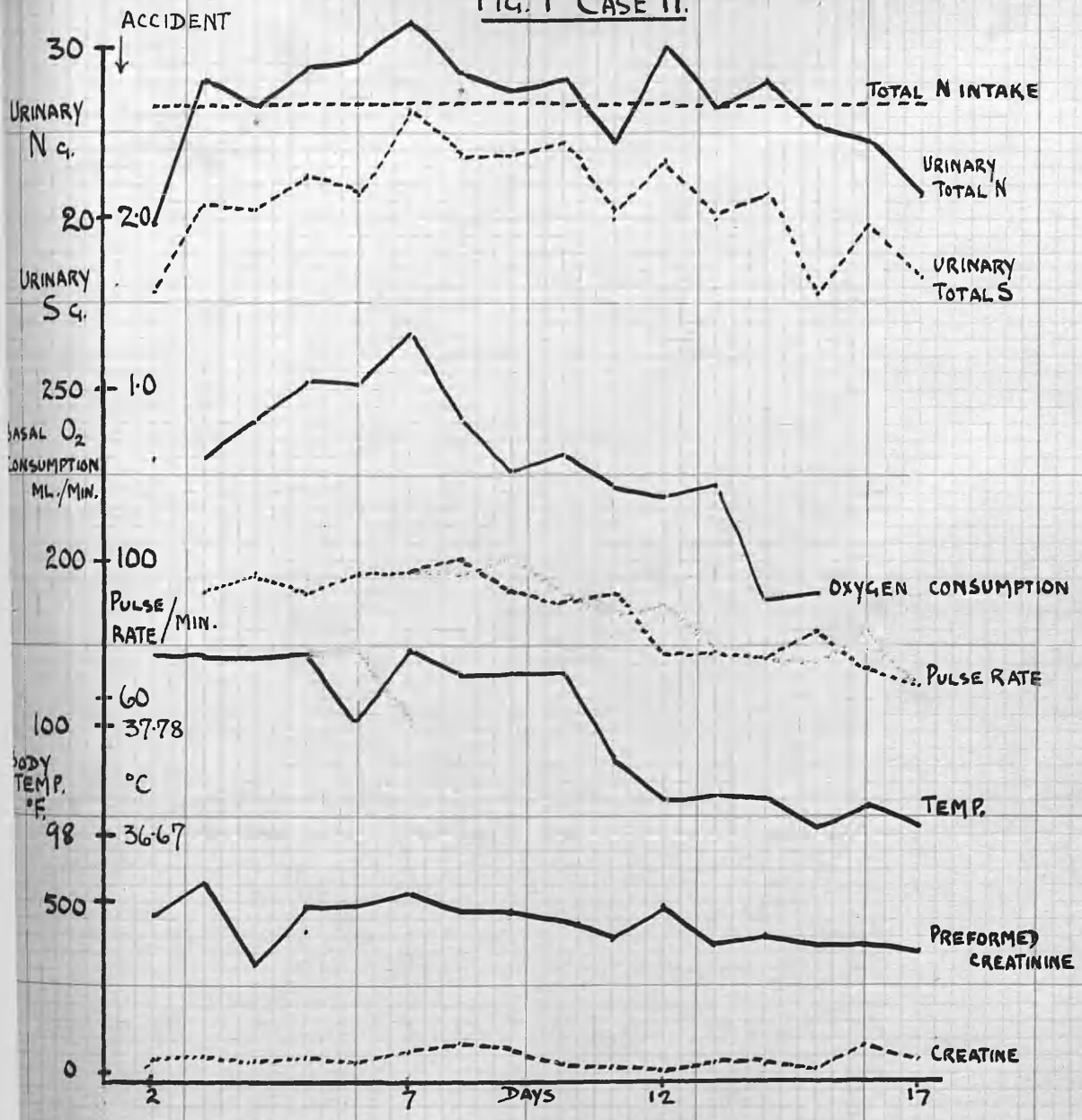
The maximum values for the basal oxygen consumption, body temperature and pulse rate coincided in time with the maximum excretions of N and S in the urine and the trend of all these values was in the same general direction - namely an irregular rise to a maximum on the seventh day and an irregular fall thereafter.

The respiratory quotient varied between 0.76-0.85 during the first twelve days, thereafter it gradually rose to 1.15.

The output of preformed creatinine was fairly constant, such variations as occurred were parallel to the general trend of N and S. The degree of creatinuria was slight and remained practically constant throughout the period of observation.

The fall of a coal tree fractured both bones of both legs of Case 12. Both bones of the right leg were fractured about the middle, those of the left leg at the junction of the proximal and second quarters. During the 10 days of observation

Fig. 7 Case II.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URINARY N g.
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TOTAL N INTAKE
URINARY TOTAL N
URINARY TOTAL S
URINARY S g.
250
1·0
BASAL O2 CONSUMPTION ML./MIN.
200
100
OXYGEN CONSUMPTION
PULSE RATE/MIN.
PULSE RATE
60
100
37·78
BODY TEMP. °F.
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TEMP.
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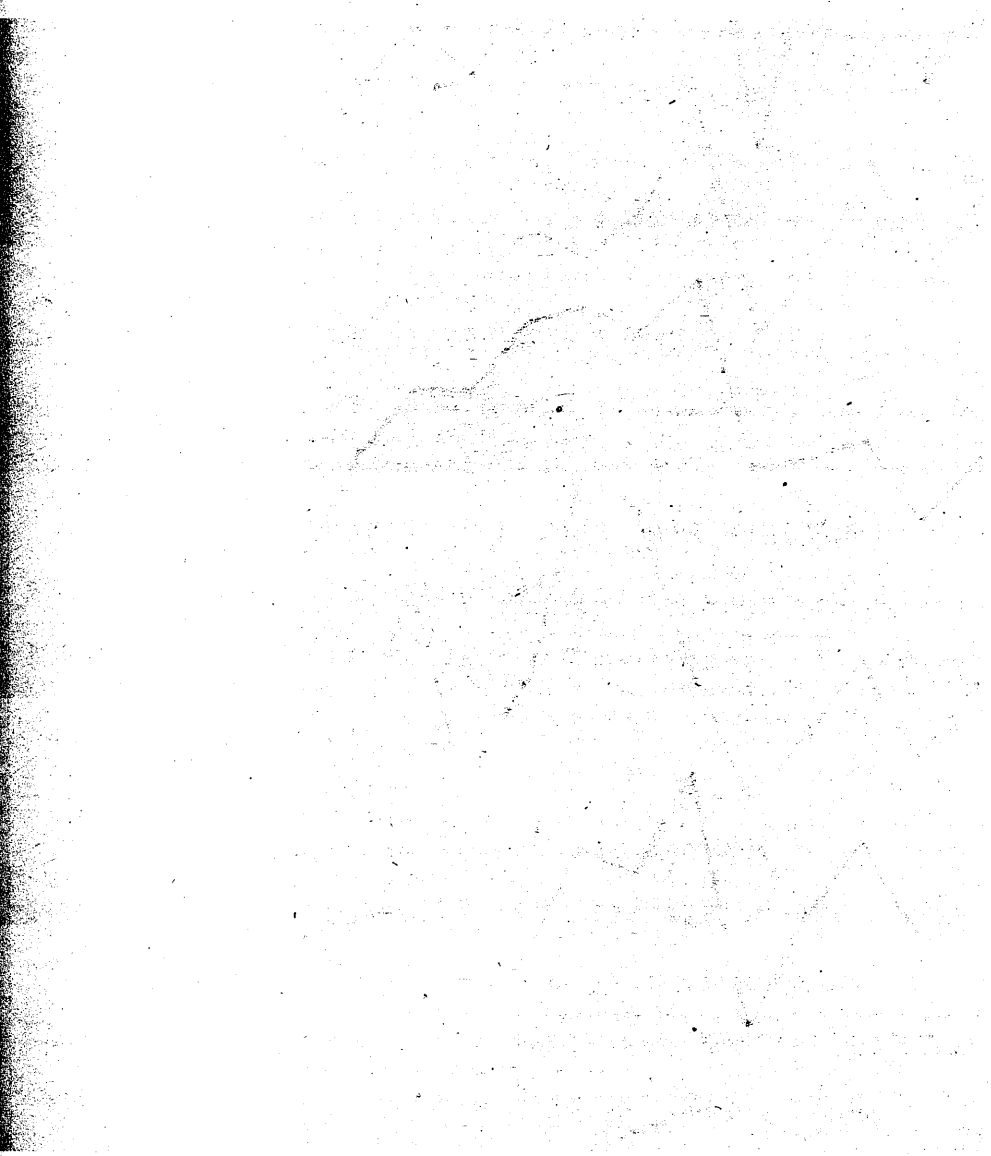
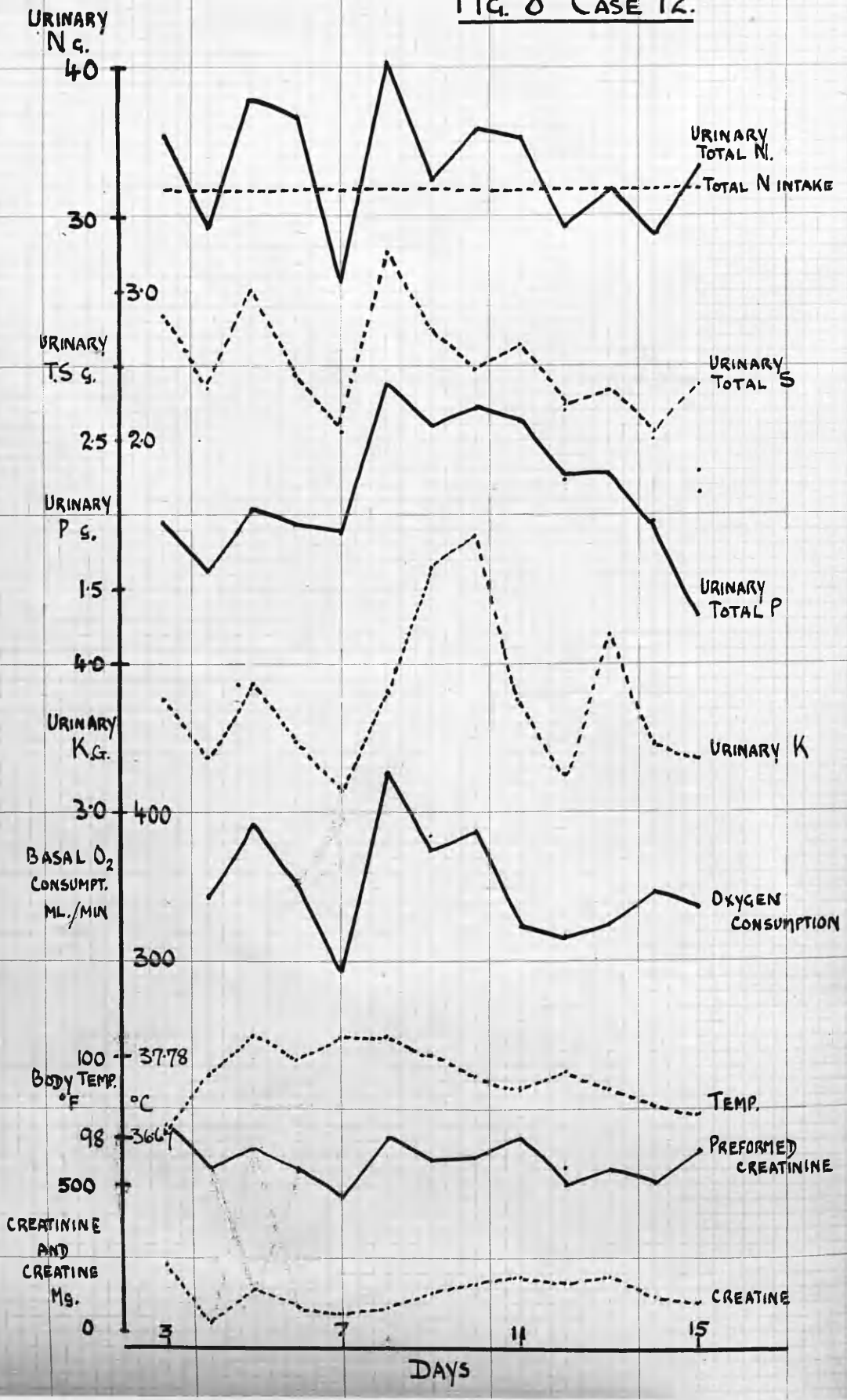


Fig. 8 Case 12.


URINARY N G.
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CREATININE AND CREATINE MG.
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CREATINE

he lost 17.59 g. N even although on a diet rich in first class protein (31.84 g. T.N. daily) and with an energy value of 3214 Cal.

The urinary volume, total N, total S, total P and to slightly less extent K exhibited similar daily variations, Fig.8. The values did not show the characteristic slow rise to a maximum and subsequent gradual fall noted in most of the other cases. The daily fluctuations in the excretion of these catabolities were paralleled by similar fluctuations in the basal consumption of oxygen, body temperature and pulse rate, indicating that these irregularities were not simply variations in the daily excretion but due to differences in the rate of metabolism.

The excretion of preformed creatinine remained remarkably constant, such fluctuations as occurred corresponded with those of the other urinary catabolities. The creatinuria gradually rose to a maximum on the eleventh day following the injury then declined.

As the result of being charged by an opponent when playing football, Case 13 fell heavily to the ground, both bones of one leg being splintered at the site of fracture (mid point). The leg was so swollen on admission to hospital that it was impossible to put it in a splint. For five days it lay between sand bags. It was then manipulated and splinted. Six days later a pin had to be passed through the os calcis and extension applied. The leg was still considerably swollen at the site of injury 16 days after the accident. During these two days of manipulation the intake of food was greatly diminished, 5 g. N being consumed on the first occasion and less than 2 g. on the second occasion.

Fig. 9 Case 13.

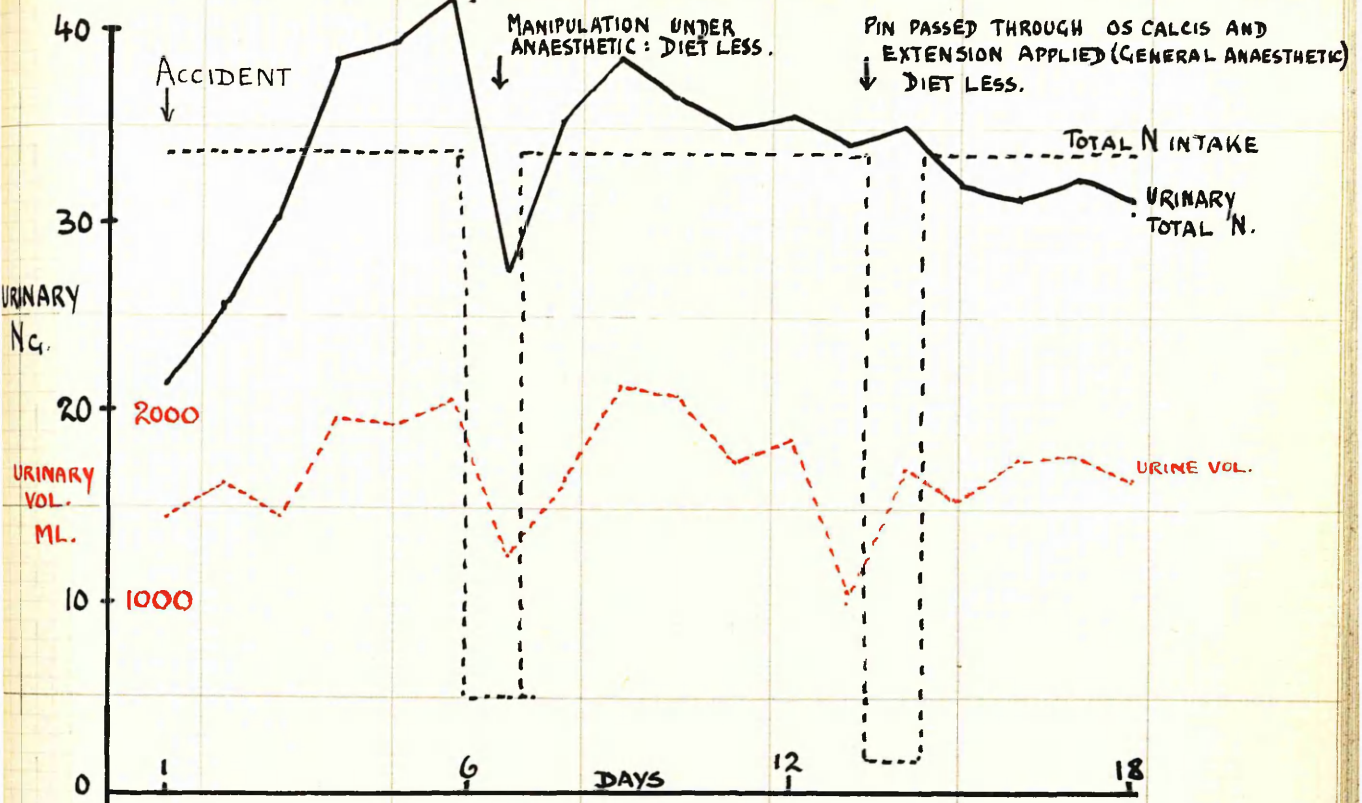


Fig. 10 Case 14.

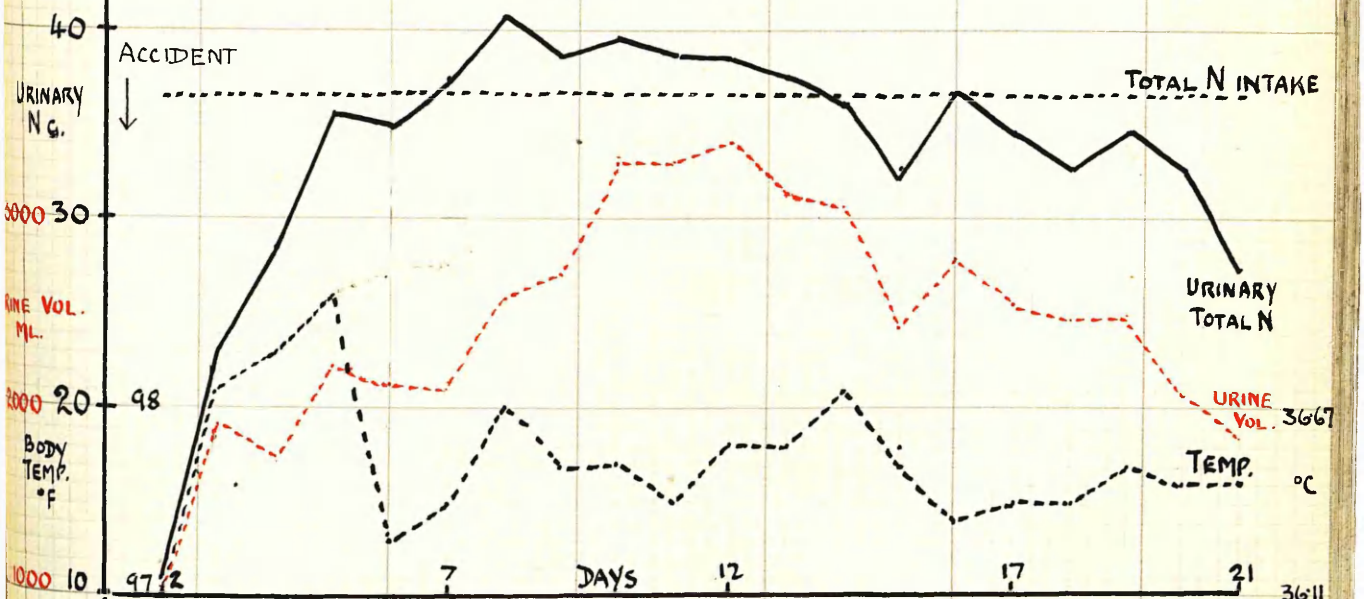
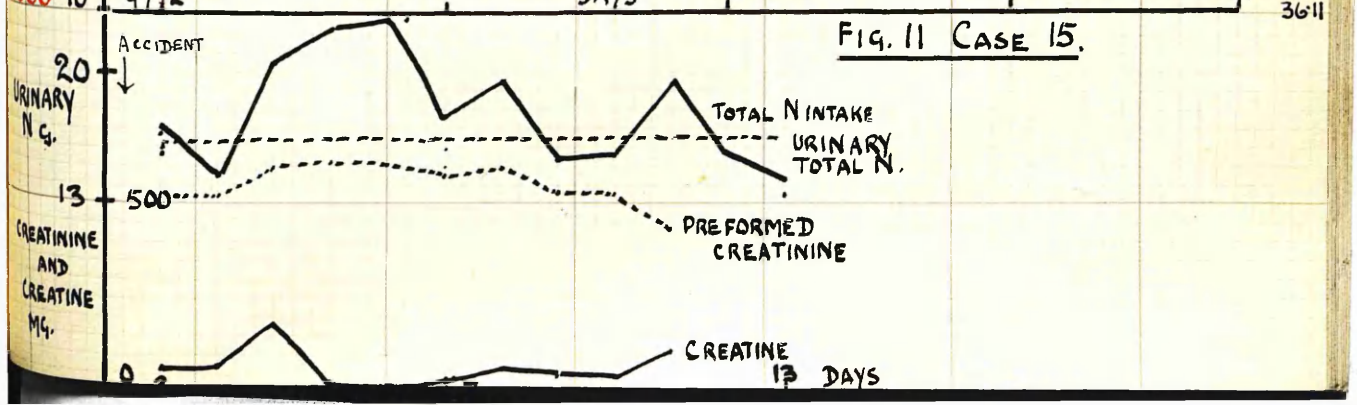


Fig. 11 Case 15.



The patient lost 45.82 g. N in the course of 15 days, despite the fact that the diet was rich in first class protein and believed to be more than adequate in energy value (3044 Cal.). The average daily intake of N inclusive of the two days of manipulation was 33.84 g. The surgical procedures each produced a definite but temporary increased excretion of N over and above the general rise and fall resulting from the original injury (Fig.9). The urinary volume and body temperature varied with the total N excretion.

Case 14, the last member in this group receiving high protein diets sustained a fracture of both bones of one leg due to a fall of stone. The daily intake of N was 36.69 g. (meat, eggs and milk mainly): the energy value 3606 Cal. During the 17 days of observation the patient lost 25.49 g. N.

From Fig.10 it appears that on superimposing such a diet, containing the maximum amount of first class protein which can be ingested over a period with a minimum of discomfort, upon a diet of average N content, and believed to be more than adequate in caloric value, there occurs first a period of retention while the N excretion is rising, then a period of loss to be followed later by another period of retention. The fact that there was a loss of N during a considerable part of the period appears to indicate, that in such cases of injury, attempts to stem completely the loss of body tissues fail during the period of maximum tissue catabolism. The maximum volume of urine was passed subsequent to that of the total N.

This group of experiments indicates that with cases of fracture due to direct violence, where in addition to the breaking of bone there is a definite amount of soft tissue damage (mainly muscle) with extravasation of blood, attempts to maintain these patients in nitrogen equilibrium during the period of maximum catabolism fail.

The recorded N losses in all the cases are of course slightly smaller than the actual since no account has been taken of the sweat N.

The effect of diets of high carbohydrate but average to low protein content.

In consideration of the sparing action of carbohydrate on the rate of protein catabolism, Cases 15 and 16 were fed with mixed diets high in energy value, carbohydrate in particular - diets of 4135 and 3592 Cals. respectively.

Case 15 had the lower end of the left fibula fractured as the result of a fall of stone. There was practically no displacement of any fragments. In the course of 9 days this man lost 35.90 g. N (daily N intake 16.39 g.)(Fig.11).

Case 16 suffered from a fracture of the lower ends of both bones of one leg. The ends were manipulated into position without anaesthesia. During the course of 5 days the patient lost 47.57 g. N (daily N intake 8.31 g.).

In both these cases the urinary volume varied with the excretion of N. The body temperatures did not rise concurrently with the total N. In Case 16 the maximum temperature (37°C) was recorded 3 days after the maximum N excretion.

Discussion.

In the course of the foregoing experiments attempts have been made to prevent the loss of N, S, etc., which occurs during the period of increased catabolism that follows severe injuries due to direct violence. In the light of the data derived from patients receiving diets rich in first class protein and of high caloric value, it is obvious that no success could have attended the earlier experiments in this series.

From certain incomplete observations on cases of fracture due to indirect violence where little or no tearing of soft tissue had resulted from the displacement of the bone fragments, the evidence suggested that N equilibrium and even N retention could be attained more easily, particularly when the cases received similar mixed diets of high protein content and energy value.

In all deep wounds such as those which result from the fracture of bone, particularly when there is marked displacement, blood vessels are destroyed and a variable amount of haemorrhage results; disturbances of a more subtle nature then take place in the regions deprived of their normal blood supply. Many cells in the path of the wounding agent or the displaced bone fragments are killed outright, while neighbouring ones are more or less seriously damaged, many succumbing to necrosis.

In the cases described in the present series, no obvious relationship could be found between the amount of damage apparently done and the catabolic response. Individuals appeared to react differently. For example the most serious injuries recorded were sustained by Case 12 (fracture of both bones of both legs).

Despite this the N loss was only 17.59 g. in 10 days when on an intake of 31.84 g. N (largely first class protein) and equivalent in energy value to 3214 Cal. The energy value of this diet was of course in excess of the patient's requirements and would naturally have led to a storage of N in the intact subject. In the light of our experience with the normal subject it may be necessary to regard the loss of N by this and other similar cases as representing a more marked catabolic response than would appear at first sight to exist.

In these fracture cases there was frequently observed a slight rise in body temperature, scarcely ever exceeding 1-2°C. It is of interest therefore to consider to what extent the fever and increased protein metabolism of these subjects could have been interrelated. Voit as far back as 1895 had shown that in fasting animals artificial pyrexia was attended by an increased protein metabolism, but that in well fed animals the protein destruction was considerably smaller. In man Schleich (1875) found that if the body temperature was raised by preventing radiation an increased N output resulted, and Linser and Schmidt (1904) showed that no breakdown of tissue protein occurred in normal persons as the result of the external application of heat, if the body temperature was maintained under 39°C. Graham and Poulton (1912-13) noted no appreciable increase in N excretion as the result of raising the body temperature to 39.44-40°C by exposure to steam heat. It is unlikely therefore, than an appreciable part of the increase in catabolism observed in these injured

subjects is due to the slight pyrexia sometimes found. It is much more reasonable to believe that the increase in temperature is (in part at least) due to the increased metabolism.

When considering the question of temperature it may be noted in passing that Ebeling (1922) found that the velocity of the reparative phenomena depended upon the rate at which certain chemical reactions took place. In the case of the alligator the reparative rate as characterised by degree of cicatrization was increased two-fold by a rise of $10^{\circ}C$. Actually the difference in environmental temperature used was $15^{\circ}C$ (23°-38°).

Several attempts have been made in the past to maintain N equilibrium at the height of an infective fever. Shaffer and Coleman (1909) believed that the lack of success lay in the fact that food of insufficient fuel value and of unsuitable character had been used. Shaffer and Coleman were, however, only able in one instance to obtain N equilibrium in the early stages of typhoid and they were unable at any time to maintain it for more than 2-3 days when the temperature was above $102^{\circ}F$ although the patients were receiving as much as 90 Cal. and 1.6 g. protein per kg. per day. Rolland (1912) almost attained N equilibrium in febrile patients with diets high in both calories and protein but Coleman and Du Bois (1915) failed to establish N equilibrium in cases of typhoid and other fevers on diets containing adequate calories and as much as 15 g. N. daily. Lauter and Jenke (1925) and others, have also failed to establish N equilibrium in fever with diets of high calorie content.

The increased protein catabolism in febrile infections

is due not only to the increased energy requirements but also to an active destruction or autolysis of tissue. The failure to attain N equilibrium at the height of catabolism in the present series of cases is all the more extraordinary therefore considering that there was little or no disturbance of body temperature.

Benedict and Surányi (1903) have denied the existence of a toxic destruction of protein in fever, believing that the increased protein catabolism is merely a part of an increase in the total metabolism, which Aronsohn (1907) held to be caused by the action of toxins on either enzymes or the nervous mechanism controlling the consumption of food and body materials. In their view, to correct the situation additional food must be given. The chief obstacle to this view is just that which has been described, namely, the apparent impossibility of preventing a loss of body N in a fevered subject at the height of his illness even though given food in excess of his supposed need. The work of Lusk (1891), Sivén (1901), Landergren (1903), Folin (1903), Murlin (1907) and others has shown the superior value of carbohydrate in sparing body protein in fever.

In those subjects on whom basal metabolic determinations were made, the oxygen consumption varied with the body temperature and pulse rate, and in most cases this corresponded to the maximum period of N excretion. This period of maximum excretion was reached from the 4th-11th day following the injury; generally the sixth day in this series. The amount of N excreted generally rose slowly to a maximum and then as slowly declined.

The excretion of total urinary S ran parallel to the curve of N excretion in the two cases 10 and 11 in which it was followed throughout the experimental period. Relative to N slightly more S was excreted in the first half of the experimental periods which lasted 10-16 days respectively. The S:N ratio of the material lost by Case 13 was 1:16.36, a ratio which suggests the catabolism of muscle substance. Although balances were not struck in the case of P, K and Na, it was apparent that the excretion of the former two elements varied with that of N, while Na remained fairly constant. The fact that K and not Na was the variable element suggests that cellular material was being catabolised, or that through increased permeability K was being lost. This important point will be dealt with in the subsequent sections.

In no case did the faecal N loss exceed 2.5 g. per day. The fluctuations in the urinary volume were mainly dependent on variations in the rate of excretion of the products of catabolism.

Omitting for the moment those cases receiving gelatin or creatine-and creatinine-containing foods, the two cases remaining (8 and 9) in whom the creatine and creatinine excretions were studied, exhibited creatinurias paralleling the changes in urinary N. The creatinine excretion tended to vary inversely with the degree of creatinuria. This was more noticeable in Case 8. The combined creatine and creatinine excretion rose and fell with the total N excretion, the fluctuations being less marked in the case of creatine alone. These increases were not due to a washing out of catabolites for the urinary volume rose to

its highest level during the period of total N and total creatinine decline. These observations suggest either, that the metabolic process which has creatinine as an end product is in part arrested at the stage of creatine, or that these two metabolites have separate origins, the creatinuria arising from the breakdown of perhaps local muscular tissue, the diminution of creatinine excretion being the result of a general decline in the activity of the total protoplasmic mass following on the injury. The latter is perhaps the more reasonable explanation.

In those cases receiving gelatin (6 and 7) the excretion of preformed creatinine was fairly constant and independent of the amount consumed. On the other hand the creatinuria was dependent, in part, on changes in the intake of this protein; for on increasing or decreasing the amount taken, similar changes occurred in the amount of creatine excreted. The level of the creatinuria in these two cases and in the one receiving glycine was the highest of the series, though never exceeding 650 mg. (in terms of creatinine N); and in those cases receiving creatine- and creatinine-containing foods (11 and 12) the degree of creatinuria was often very constant and generally of a lower value than was observed in the other cases.

III. The effect of fracture of bone on the metabolism of the rat with observations on the result of feeding additional carbohydrate.

At this point a digression will be made to relate some experiments performed on the rat which have an intimate bearing on the metabolic response to injury. In the prosecution of this part of the research I have been greatly indebted to my colleague Dr. J. Sloan Robertson and to Mr. J. McGirr an honours student in this Department. Mr McGirr was responsible for the the potassium and sodium analyses.

The problem was to determine the relative importance of the following three possible sites of increased tissue catabolism in contributing to the negative N, etc., balance.

1. Damaged tissue in process of removal for repair purposes.
2. Atrophy of the uninjured muscles of the injured limb, which clinical observation shows to be a constant sequel to injury but which is very variable in degree.
3. General increase in protein catabolism of the tissues, muscle in particular.

(1) is an essential feature of repair and must always occur. (2) would most probably result from a local spinal reflex, as suggested by the work of Harding (1925, 1926 and 1929). (3) might be a more general spread of this reflex, or be due to the presence in the blood stream of the products of the breakdown of the damaged tissue.

It is noteworthy that clinical experience testifies to the more rapid and severe muscular wasting which follows injury to a well innervated structure like a joint, than follows a

similar degree of trauma to a less well innervated organisation like bone. Clinically any method which could abolish (2) and (3), but especially (2), would be of great value in shortening convalescence. The present work is a note of certain preliminary experiments to determine the part played by each factor.

Experimental.

Standard young adult male rats of the Rowett Research Institute albino strain, weighing 300-350 g. were housed in metabolic cages somewhat similar in essential design to the Hopkins metabolic cage. Urine and faeces were separated by Hopkins' arrangement. The animals were fed Thomson's dried powdered composite food (Thomson, 1936) plus a ration of lettuce (first series) or cabbage (second series) and an unlimited water supply.

The dried food stuff consists of:

	%
Wheat offal (fine middlings No.2)	19.2
Ground wheat	19.2
Sussex ground oats	19.2
Ground barley	9.5
Ground maize	9.5
Meat and bone meal (45% protein)	9.5
Dried skimmed milk	7.0
White fish meal (60% protein)	4.7
Dried yeast (40% protein)	1.2
Sodium chloride	0.5
Cod-liver oil	0.5

A preliminary examination in which two animals were fed an unlimited amount of this diet indicated that appetite failed after operation, the animals only partaking of a fraction of their previous intake. During the preperiod they had put on weight.

TABLE 1.

Daily intake of food.

	Rat 2.		Rat 3.
	18.03 g.		12.45 g.
	17.68 g.		15.08 g.
	17.80 g.		15.75 g.
Ether anaesthesia,	18.84 g.		14.93 g.
open fracture +	→ 5.77 g.	→	1.53 g.
trauma to muscle	11.16 g.		7.51 g.
	10.77 g.		13.26 g.
	10.57 g.		13.82 g.
	12.23 g.		15.22 g.
	14.34 g.		16.66 g.
	14.67 g.		16.25 g.
	14.29 g.		19.18 g.

To assess the result of trauma on N metabolism control animals fed the same rations were used. This method proved unsatisfactory however and it was resolved to place the animals on constant diet just sufficient to maintain body weight. This proved satisfactory for on only one occasion did appetite fail in the period following the operation. Part of this success was due to performing the operation early in the day appointed, so that time was allowed for the animal to recover from the anaesthetic and shock and regain its former eating habits within the 24 hours. Under such dietary conditions it became obvious that during the convalescent period the animal's dietary requirements had increased considerably.

Each morning at a fixed hour the collecting glass funnels were washed down with dilute H_2SO_4 (circa 0.5 per cent) and about

15 ml. of this dilute H_2SO_4 were placed in the collecting beaker-flask. A test for the adequacy of this method of collection was carried out as follows. An aliquot of a rat's urine diluted 1:1 and of known T.N. content was sprayed over the collecting funnel to imitate the distribution of urine by the animal. After an interval of 48 hours the collecting funnel was washed down with very dilute H_2SO_4 and the N content of the fluid collected was determined after filtration. The original urine contained 1.624 g. N per 100 ml. diluted urine: the fluid collected contained N equivalent to 1.579 g. per 100 ml. of the original diluted urine - a loss of 2.7%. This loss was considered negligible when consideration was taken of the point at issue.

A slight amount of the dried food was scattered but never amounted to more than a small fraction of a gramme. It was filtered off prior to analysis. In order to determine if a significant amount of N could be leached out of this food by the weak acid during the process of washing and filtration, 1 g. of the food was extracted with dilute H_2SO_4 overnight and then analysed. The N extracted amounted to 0.00151 g., a negligible amount.

The urines were made up to 200 ml. after filtration and thorough washing of the collecting vessels. Thymol in chloroform was used as a preservative.

Methods: N in urine, faeces and food - Kjeldahl method.

Creatine and creatinine - micro method of Folin (1914).

A creatinuria was constantly observed in normal and injured animals and a subsequent experiment indicated that over

a period of 5 days no transformation of creatine into creatinine took place in the urine on standing.

A N balance experiment was carried out on Rat 1, there being no previous operative procedure on that animal.

Urinary T.N. for period of 4 days	1.708 g.
Faecal T.N. for period of 4 days	0.387 g.
	2.095 g.
Intake of N for period of 4 days (lettuce excluded)	2.189 g.
+ Balance	0.094 g.
Apparent retention of N per day =	0.0235 g.

The hair loss during the period was not estimated.

This balance experiment was considered satisfactory as indicating that weight equilibrium was coincident with N equilibrium in the preperiod.

The experiments now to be described really form two groups both in point of time and in experimental procedure. Each group has been divided into several series. In all instances the general anaesthetic used was 'open' ether.

GROUP I.

Preliminary series (Rats 2-4).

Following a preliminary preperiod two rats were epilated over the outer aspect of the left thigh, and an open comminuted fracture of the left femur produced about the junction of lower third with upper two thirds. The wound was sutured and the limb not splinted. Within a few hours the animals were found to be moving

about the cage and appeared to suffer very little inconvenience as the result of the fracture, the advantage of using a four-legged animal. For each animal a control originally comparable in weight and dietetic demands was selected and fed an intake corresponding to that eaten by the operated animal the day previously. Open ether anaesthesia was given for a comparable time on the day corresponding to the operation day of its vis-à-vis. Unfortunately an accident to one control rendered the data obtained from Rat 2 of little use.

During the preperiod these animals were on an unrestricted diet as regards the quantity of food eaten.

Second Series:

The animals in this series were placed on a constant diet just sufficient to maintain an approximately constant body weight. They were divided into 3 lots.

Lot 1 - Open fracture as in first series. (Rats 5 and 6.)

Lot 2 - Incision as in Lot 1 with corresponding initial amount of muscle damage but no fracture. (Rats 7 and 8.)

The extensor muscles were damaged by squeezing those just above the point corresponding to the fracture site in other animals, with the bone crushing instrument used. Full pressure was not employed. This amount of muscle damage can only simulate in a very rough manner the damage caused by the over-riding jagged bone ends.

Lot 3 - As Lot 1, but with introduction of a local anaesthetic consisting of ethyl p-amino benzoate and benzyl esters. 0.3 ml. in oily solution were injected into fracture area at the time of the operation. (Rats 9 and 10.)

Local anaesthesia is frequently used clinically to facilitate reduction of fractures but with no other intent.

Third Series: (Rats 9 and 11.)

During the last 5 days of the post-fracture period of Rat 9, 5g. additional carbohydrate, as cane sugar, were added to the diet. Rat 11 also received additional carbohydrate in the post-fracture period but in this case from the day of operation. In this animal the basal diet of the composite food was fed as usual in the morning at 9 a.m. If this had all been eaten by 2 p.m., as was nearly always the case, cane sugar was added in an amount sufficient to restore weight. This was found by observation to be approximately gramme for gramme. At 5 p.m. the additional carbohydrate was removed and the amount not eaten, if any, recorded. With this procedure the animal was eager for its basal diet the following morning.

At the close of the experimental periods the animals were killed with ether and weighed. The skin over the lower half of the trunk and hind limbs was removed. The hind limbs were removed each with half of the pelvic girdle, the latter being cut in a symmetrical manner. As it was found difficult to remove all the muscle groups around the fractured femur, the knee extensor group (equivalent to the quadriceps femoris) only, was removed after stripping off the sartorius. The loss in weight of this group of extensor muscles, when compared with the corresponding muscle group of the contra-lateral limb, was used as an index of the effect of the fracture on the adjacent muscle groups. The wasting of the flexors of the knee was not estimated as accurate dissection of these seemed impossible.

DISCUSSION ON DATA DERIVED FROM FIRST GROUP OF RAT EXPERIMENTS.

Body and Muscle Weight Changes (Table 2).

Apart from Rat 11 all the other rats with fractured femora lost weight. As will be seen from Table 2 this loss in weight was considerably greater than could be accounted for by the loss in weight of the injured limb. The loss in weight of the injured limb was greater than the loss in weight of the locally damaged muscles as judged from the wasting of the knee extensor group, except in the case of the two animals (Rats 9 and 10) which received a local anaesthetic into the fracture area. In these animals the loss in weight of the extensor group accounted for as much as two thirds of the loss in weight of the half pelvis plus limb. The remaining one third could probably be accounted for by the wasting of the other muscle groups around the fracture area.

The two control animals (Rats 7 and 8) with incision to bone and muscle damage, also lost weight. The period of observation on these animals was shorter than on the fractured animals. If the extent of the weight losses over comparable periods of time be taken, it is found that there is little if any significant difference between the two groups. No loss of weight of the injured limb was noted in one animal but in the other a difference of 1.165 g. was found, less than half that observed in the animals with fracture but without local anaesthetic. The weight change in the knee extensor group of muscles was least in this series.

TABLE 2.

Expt. No.	Nature of Experiment	Time interval between day of operation and post-mortem exam. Days.	Loss in weight of animal. g.	Loss in weight of injured limb ($\frac{1}{2}$ pelvis + limb). g.	Loss in weight of injured quadriceps femoris. g.	Average daily urinary T.N. of preperiod. mg.	Average daily N balance per 5 day period dating from day of operation. mg. N.				Total N loss over period (days) mg.	N equivalent of weight lost by animal on basis of 3 g. N ≡ 100g. 'flesh'. mg.	N equivalent of weight lost by $\frac{1}{2}$ pelvis + limb on basis of 3 g. N ≡ 100 g. 'flesh'. mg.
							1-5	6-10	11-15	16-20			
3	Open fracture - diet uncontrolled.	9	19	2.99	0.96	-	-47 (When compared with control)	-25	-	-	336	633	99
4	Control for 1.	9	6 (Gain)	-	-	-	-	-	-	-	-	-	-
5	Open fracture - constant diet.	14	33.5	2.785	0.45	341	-79	-71	-30 (11-14 days)	-	870	1117	93
6	Open fracture - constant diet.	19	26	2.74	0.98	354	-73	-30	-64	+ 39 (16-19 days)	679	866	91
7	Incision to bone + muscle damage constant diet.	7	22.5	1.165	0.20	300	-57	-14 (6-7 days)	-	-	313	730	39
8	Incision to bone + muscle damage - constant diet.	7	17	0.14 (Gain)	0.37	378	-53	-1 (6-7 days)	-	-	267	566	-
9	Open fracture + local anaesthetic - constant diet with exception of last 5 days.	17	22.5	0.95	0.62	341	-81	-71 (6-12 days) (7 days)	+ 17 (13-17 days) (extra carb.)	-	817	730	32
10	Open fracture + local anaesthetic - constant diet.	14	40	0.90	0.63	353	-76	-48	-22 (11-14 days)	-	730	1333	30
11	Open fracture + extra carbohydrate.	15	10 (Gain)	2.41	0.72	386	-28	+ 3	+ 45	-	+100	+333	80

The addition of extra carbohydrate in the form of cane sugar to Rat 9 during the concluding days of the experiment retarded the fall in body weight which had been in progress from the day of operation, and indeed caused a rise in weight. The addition of extra sugar to the diet of Rat 11 prevented this fall in weight which appears to be the natural concomitant of injury when applied to an animal on a constant basal diet just sufficient to maintain body weight. On the two occasions when the additional cane sugar intake was removed a fall in body weight resulted. Resumption of the extra carbohydrate restored the weight. Although the body weight of this animal was maintained constant, comparison of the weight of the injured limb and its extensor group revealed that reflex wasting of the limb had not been prevented by the additional carbohydrate but simply the more general loss of body tissue.

'Open' ether anaesthesia *per se* was found to have no appreciable effect on body weight or protein metabolism.

The information derived from these observations on the loss of weight of the whole animal, the injured limb and its quadriceps femoris led to the conclusion:

(1) that a loss of weight always occurs in animals subsisting on the diet which prior to the injury was just sufficient to maintain body weight,

(2) that the wasting of the injured limb and its quadriceps femoris in the fractured animals is greater than those which would result from the operative procedures *per se* (i.e. excluding the actual fracture of bone),

(3) that the wasting of an injured limb can apparently, in part at least, be diminished by the action of a local anaesthetic with a prolonged action such as produced by a mixture of ethyl p-amino benzoate and benzyl esters in an oily basis;

(4) that local anaesthesia does not diminish the loss in weight of the damaged muscles as such damage is due to the relatively unrestricted tearing action of the jagged bone ends during the early stage of healing. Part of the loss in weight of the extensor muscles is reflex in origin, part due to the absorption of the damaged tissue. The local anaesthetic if it possesses a prolonged action should diminish the wasting due to reflex action if pain be the cause;

(5) that these local effects (reflex and absorptive) cannot be mitigated by additional carbohydrate to the diet, but that the general loss in weight can very definitely be prevented by extra calories. This general loss in weight must be due to an accelerated catabolism in which tissue protein plays a part.

Total Urinary N (Tables 2-9, Figs. 1-7).

As the result of the operative interference the output of N in the urine was definitely increased, there being in consequence a negative N balance. This confirms our previous observations on the human subject.

The reaction to muscle injury without fracture was over in one to five days but that due to fracture lasted for a

TABLE 3.

Rat 5. (Basal Diet 15g.)

Day of diet.	Diet.	Body Wt g.	Total N mg.	Total Creatinine mg.
8	Basal	327	331	
9	"		357	
10	"		368	22
11	"	334	364	23
Op.*				
12	"		384	25
13	"		400	26
14	"		468	33
15	"		437	29
16	"	321	413	27
17	"		402	27
18	"		433	29
19	"	310	416	30
20	"		403	26
21	"		407	27
22	"	307	425	28
23	"		366	24
24	"	298	406	23
25	"		286	18
P.M.26	"	300.5		

* Op. = operation.

TABLE 4.

Rat 6. (Basal diet 15 g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	Total Creatinine mg.
13	Basal	340	361	21
14	"		343	23
15	"	343	359	26
Op.				
16	"		455	30
17	"		445	28
18	"		461	32
19	"	332	441	29
20	"		352	24
21	"		450	32
22	"	320	324	21
23	"		493	39
24	"		416	25
25	"	314	259	25
26	"		450	27
27	"	304	428	27
28	"		436	27
29	"	304	460	27
30	"		336	21
31	"	315	323	23
32	"		298	22
33	"		334	22
P.M.34	"	317	320	20

TABLE 5.

Rat 7. (Basal diet 13 g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	Total Creatinine mg.
16	Basal		317	
17	"	293	305	
18	"		273	
19	"	292	298	
20	"		287	20
21	"	296	284	19
22	"		319	23
23 Op.	"	298	319	23
24	"		362	28
25	"		424	30
26	"		334	25
27	"	282	308	22
28	"		360	27
29	"	282	316	24
30	"		312	23
P.M.31		275.5		

TABLE 6.

Rat 8. (Basal diet 15 g.)

Day of diet.	Diet.	Body Wt. g.	Total N mg.	Total Creatinine mg.
11	Basal		414	
12	"		347	
13	"	332	368	
14	"		387	
15	"		381	
16	"	339	371	
17	"		459	
18	Reduced intake.		260	
19	Basal		348	25
20	"	338	287	19
21	"		343	24
22	"	333	365	25
Op.				
23	"		542	46
24	"		491	35
25	"		384	23
26	"	322	396	26
27	"		341	24
28	"	320	359	25
29	"		400	26
P.M.30	"	316		

TABLE 7.

Rat 9. (Basal diet 15 g.)

Day of diet.	Diet.	Body Wt. g.	Total N mg.	Total Creatinine mg.
6	Basal		289	
7	"	341	303	
8	"		363	21
9	"		338	25
10	"	351	312	22
11	"		342	22
12 Op.	"	345	348	23
13	"		413	32
14	"	343	391	28
15	"		427	24
16	"		459	29
17	"		420	29
18	"	334	397	31
19	"		389	30
20	"		437	31
21	"	324	407	30
22	"		401	28
23	"	319	421	30
24	"		434	29
25	" + 5g. CHO	314	367	26
26	" " "		342	22
27	" " "	322	280	20
28	" " "		315	22
P.M.29	" " "	322	315	21

TABLE 8.

Rat 10. (Basal diet 14 g.)

Day of diet.	Diet.	Body Wt. g.	Total N mg.	Total Creatinine mg.
5	Basal	308	294	
6	"		298	24
7	"		406	24
8	"		352	25
9	"	316.5	364	21
10	"	316	403	24
Op.				
11	"		427	31
12	"	308	423	30
13	"		420	26
14	"		307	18
15	"		567	37
16	"	294	418	28
17	"		365	25
18	"		456	32
19	"	289	376	28
20	"		388	29
21	"	284	395	27
22	"		390	26
23	"	278	302	20
24	"		414	28
P.M.25		276		

TABLE 9.

Rat 11. (Basal diet 16 g.)

Day of diet.	Diet.	Body Wt. g.	Total N mg.	Creatinine mg.	Creatine mg.
6	Basal		355	14	5
7	"	304	373	14	8
8	"		366	12	10
9	"	291	419	13	13
10	" + 2 g. CHO	290	390	13	14
11	" " "	290	372	13	12
12 Op.	" + 3 g. CHO		401	14	13
13	" " "		264	8	11
14	" + 5 g. "	296	520	16	19
15	" " "	301	503	16	19
16	" + 10 g. "	305	352	14	12
17	Basal	291	430	14	12
18	" + 8 g. CHO	300	425	17	14
19	" + 4 g. "	299	348	14	9
20	" + 8 g. "	299	356	13	11
21	" " "	298	336	12	12
22	Basal	293	448	16	14
23	" + 8 g. CHO	299	302	13	11
24	" + 4 g. "	300	368	14	8
25	" " "		330	12	10
26	" " "		306	12	7
P.M. 27	" " "	300	400	15	12

FIG. 1 RAT 5

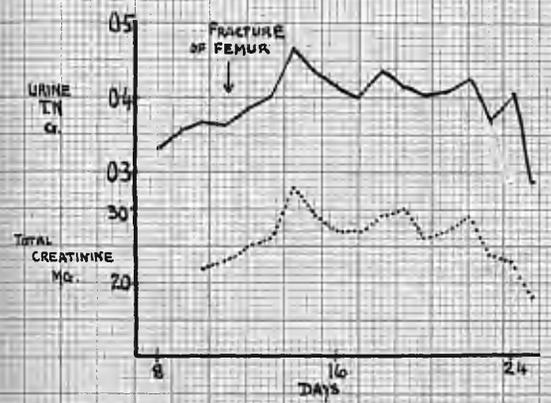


FIG. 2 RAT 6.

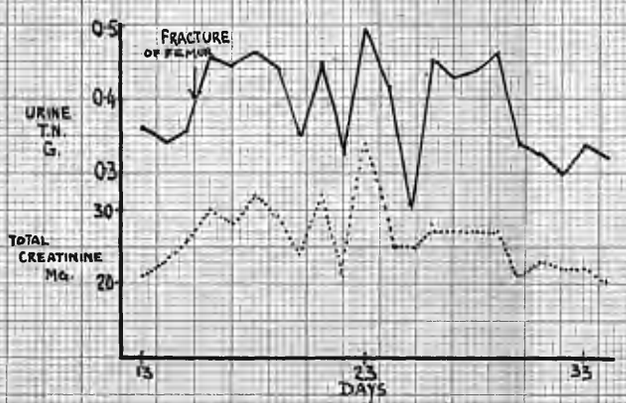


FIG. 3 RAT 7.

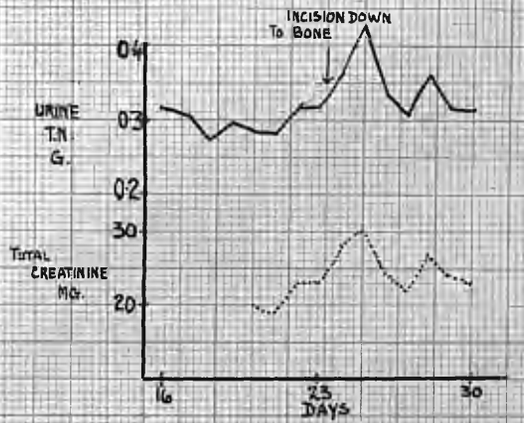


FIG. 4 RAT 8.

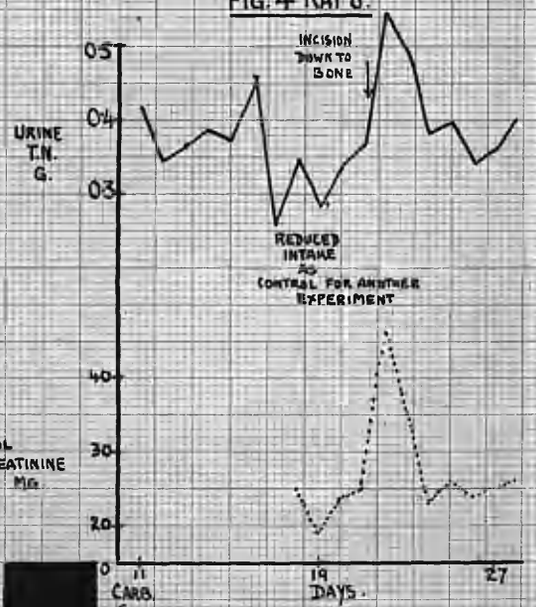


FIG. 5 RAT 9.

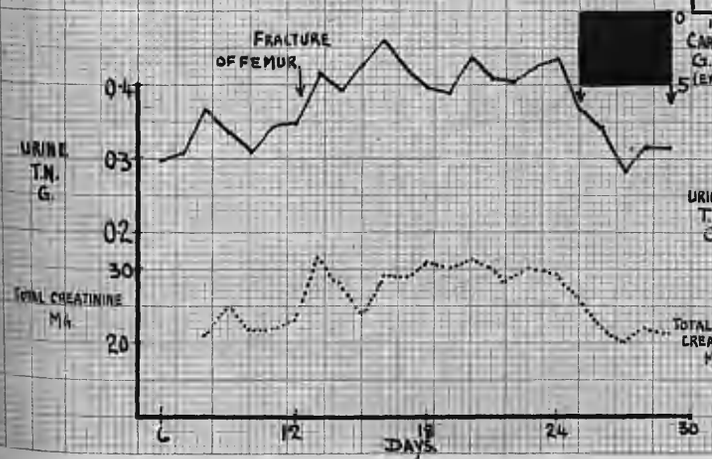


FIG. 6 RAT 10.

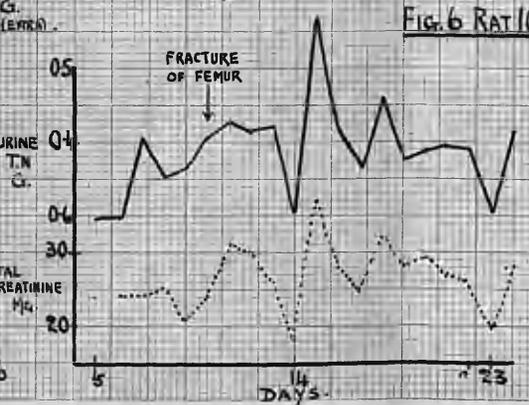
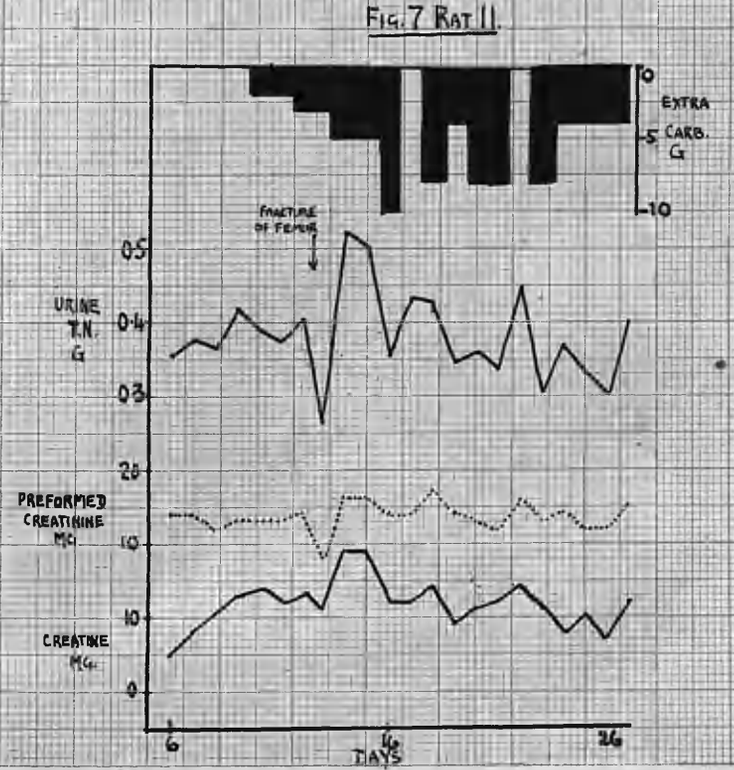


FIG. 7 RAT 11.



considerably longer period of time. In one animal the urinary N did not decline to the basal value till the fifteenth day following the operation. The prolonged N loss by these animals was probably, in part, due to slight undernutrition.

The total N loss over the experimental period was determined for each animal, and compared with the N equivalent of the weight lost by the animal and the N equivalent of the weight lost by the injured limb. In this calculation it was presumed that the total loss of weight in each case was muscle and that all the components of the muscle took part in the process, there being a proportionate loss of all the muscle constituents. The basis for the calculation was 3 g. N equivalent to 100 g. muscle. The extent to which this assumption is justifiable will be considered later.

It is obvious from the second and third last columns of Table 2 that the actual N loss was only one half to two thirds of the calculated loss on the basis that the weight change was chiefly due to a loss of muscle substance. The most probable explanation is that muscle tissue is sharing in a more general catabolic disturbance which is also involving the fat reserves. On the other hand, it may be that the difference between actual weight loss and that calculated on the N basis is due to a fluid shift. The volume of urine passed by these animals was not measured owing to technical difficulties.

On a diet just adequate for the maintenance of body weight the additional energy requirements are obtained by the utilisation of the carbohydrate, fat and protein reserves. This

is a general reaction and obviously unlikely to be diminished by the application of a local anaesthetic to the injured site. The addition of extra carbohydrate presumably spares the tissue protein and fat reserves. The carbohydrate reserves are probably exhausted very early in this phase of increased metabolism. It is obvious that the addition of carbohydrate or any other source of energy is unlikely to influence the loss due to the local spinal reflex which causes atrophy of muscles. Neither can local anaesthesia nor additional calories alter the changes undergone by the lacerated and otherwise injured muscle. This is only natural for these changes are intimately bound up in the healing process.

It is of interest to note in passing that when the animal is allowed a free choice of the quantity of food, it should select a reduced intake on the days following the injury. This is probably due to the fact that during the pre-operation period these animals were slowly increasing in weight and had perhaps therefore a greater reserve than their fellows. The writer's clinical experience is in accord with this loss of appetite following trauma.

Despite the additional energy (12-40 Cal.) supplied by the cane sugar in these rat experiments there was a loss of tissue N during the first five days following the operation on Rat 11.

Creatine and Creatinine (Tables 3-9, Figs. 1-7).

The total creatinine output was found to parallel the fluctuations in the urinary N level. In the case of Rat 11 both creatine and preformed creatinine were determined. Both catab-

olites were present in the urine in approximately equal amount. Such a high degree of creatinuria was somewhat unexpected and indicated that the animals were possibly suffering from slight undernutrition, for presumably these adolescent animals would have continued to grow at a faster rate, if their body weight increase had not been retarded by restricting the diet to one just sufficient to maintain the body weight.

GROUP 2.

As the evidence supporting the foregoing conclusions was rather meagre it was felt that some further confirmation should be obtained by investigating a further batch of rats. The animals selected were approximately of the same age as the previous group and obtained from the same breeding station. This group was piebald; the previous lot albino. The animals were provided with a slightly higher food intake than the previous group, the nature of the food being the same, except that cabbage was substituted for lettuce. The routine procedures were similar to those observed in the first group except that the animals were slain on the morning of the ninth day following the fracture.

Prior to the commencement of the experiments three normal animals were killed, so that the difference in weights between the right and left hind quarters and knee extensor group of muscles might be compared.

Rat No.	Difference in hind limbs: Right - left.	Difference in quadriceps femoris Right - left.
12	0.35	0.10
13	0.47	0.04
14	0.18	0.05

These weighings afford an index of the accuracy with which the various dissections could be performed. It must be noted that the removal of the quadriceps femoris from the injured animal's hind limbs was not so easily performed as in the normal, for a certain amount of adhesion had usually taken place between the fibrous covering of the jagged bone ends and the overlying muscle. A point of interest lies in the finding that the right hind limb and also its quadriceps femoris were heavier than those of the corresponding side, and this in a four footed animal!

In most of the experiments the urine was analysed daily for total N, creatinine, creatine, potassium and sodium. The muscles of certain of the animals were analysed for total N and K.

The animals were divided into five series.

First series: Rats 15 and 16 (Tables 10-11, Figs.8-9).

The left femur of each of these animals was fractured under 'open' ether anaesthesia when the N and K excretions were relatively constant. Appetite did not fail as the result of the operation.

TABLE 10.

Rat 15. (Basal diet 15 g.)

Day of const. diet.	Body Wt. g.	Total N. mg.	K mg.	Na mg.	Creatinine mg.	Creatine mg.
1	301					
4	301	337	70	360	-	-
5	-	334	78	380	10	7
6	301	352	70	353	11	9
7 Op.	-	342	71	355	11	7
8	305	377	78	373	10	7
9	-	373	91	364	11	10
10	297	422	100	360	10	14
11	-	386	95	356	9	11
12	287	365	86	365	8	12
13	-	348	77	355	10	12
14	288	361	76	362	10	6
15	-	331	70	352	8	6
P.M.16	-	-	-	-	-	-

Fig. 8 Rat 15.

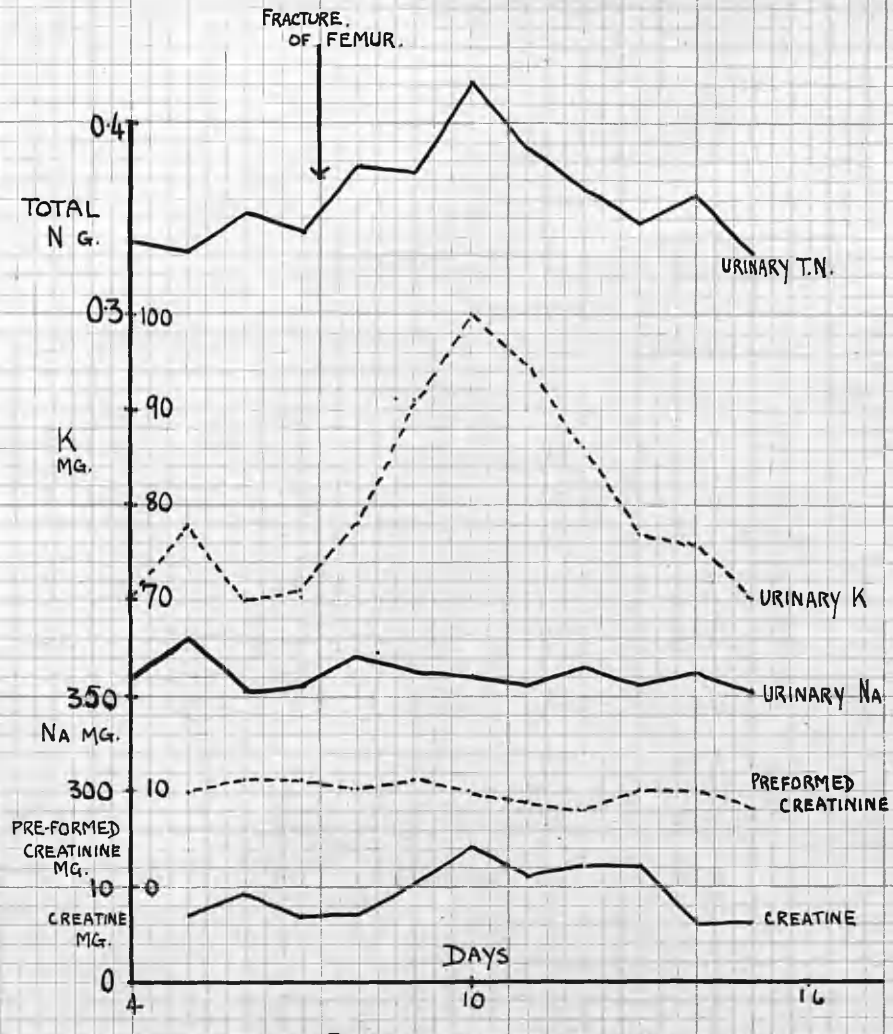


Fig. 9 Rat 16

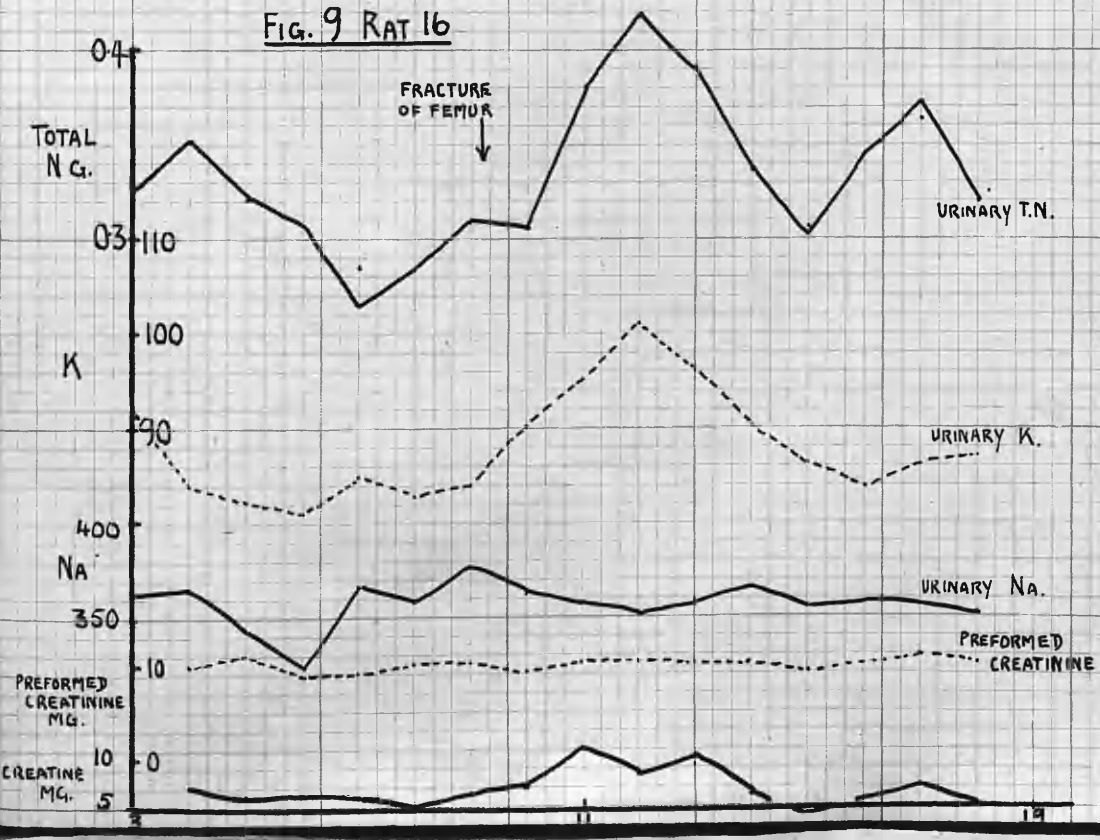


TABLE 11.

Rat 16. (Basal Diet 15 g.)

Day of const. diet.	Body Wt. g.	Total N. mg.	K mg.	Na mg.	Creatinine mg.	Creatine mg.
1	299					
3	-	327	92	363	-	-
4	299	352	84	366	10	7
5	-	321	82	343	11	6
6	298	307	81	323	9	6
7	291	267	85	366	9	6
8	-	284	83	359	10	5
9	302	311	84	376	10	6
Op.						
10	-	307	90	363	9	7
11	300	380	95	358	10	11
12	-	418	101	350	10	8
13	300	390	96	356	10	10
14	-	338	90	365	10	6
15	298	304	86	355	9	4
16	-	345	84	358	-	-
17	302	372	86	355	11	7
P.M.18	292	320	87	351	10	5

Following the injury the excretions of total N, creatine and K rose reaching their maxima on the third day following the operation. The Na and creatinine excretions remained remarkably constant. A secondary small rise in the elimination of the variable metabolites occurred on the 7th day with Rat 15 and reached a maximum on the 8th day in the case of Rat 16.

The excess outputs of N, creatine and K over the calculated basal for the same period were for Rat 15, 235 mg., 14 mg., and 97 mg. respectively and for Rat 16, 384 mg., 10 mg., and 59 mg. respectively. The local loss of tissue by the injured hind limb could not account for the excess output of N and K.

Second series: Rats 17 and 18 (Tables 12-13, Figs. 10-11).

The procedure adopted was identical to that used with Rats 15 and 16 except that 0.4 ml. local anaesthetic (Allen and Hanbury's 'A.B.A.') was injected at the time of the fracture, in the hope that its anaesthetic action would be prolonged and would reduce the reflex wasting in the injured limb as it appeared to do in the first group of experiments. The loss of weight sustained by the injured limb of Rat 17 was however the largest found in this group of experiments amounting to 2.86 g. Faulty bisection may possibly have been the cause in this instance. It is to be noted that in this group of experiments, frequently very little difference was found between the weights of the normal and injured hind quarters, particularly if it be granted that the right limb tends to be heavier than the left. It is necessary therefore, to revise our previous statement that this type of

TABLE 12.

Rat 17. (Basal Diet 16 g.)

Day of const. diet.	Body Wt. g.	Total N mg.	K mg.	Creatinine mg.	Creatine mg.
1	333				
3	334	361	88		
4	-	402	88	11	5
5	332	402	86	13	7
6	-	380	86	11	5
7 Op.	334	377	91	11	7
8	-	392	92	11	8
9	325	450	99	12	11
10	-	481	104	13	12
11	320	452	109	11	12
12	-	396	102	10	9
13	295	412	92	10	11
14	-	426	91	11	12
15	322	391	86	11	6
P.M.16	316.5	359	86	11	4

FIG. 10 RAT 17.

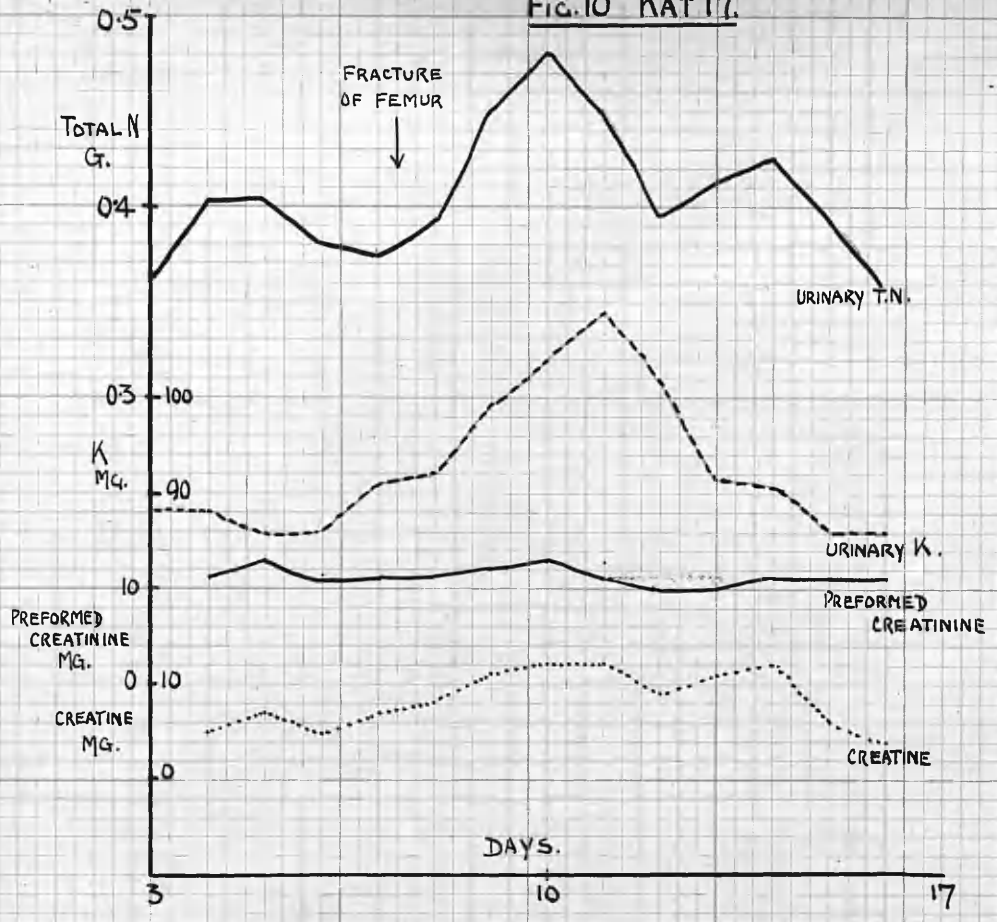


FIG. 11 RAT 18.

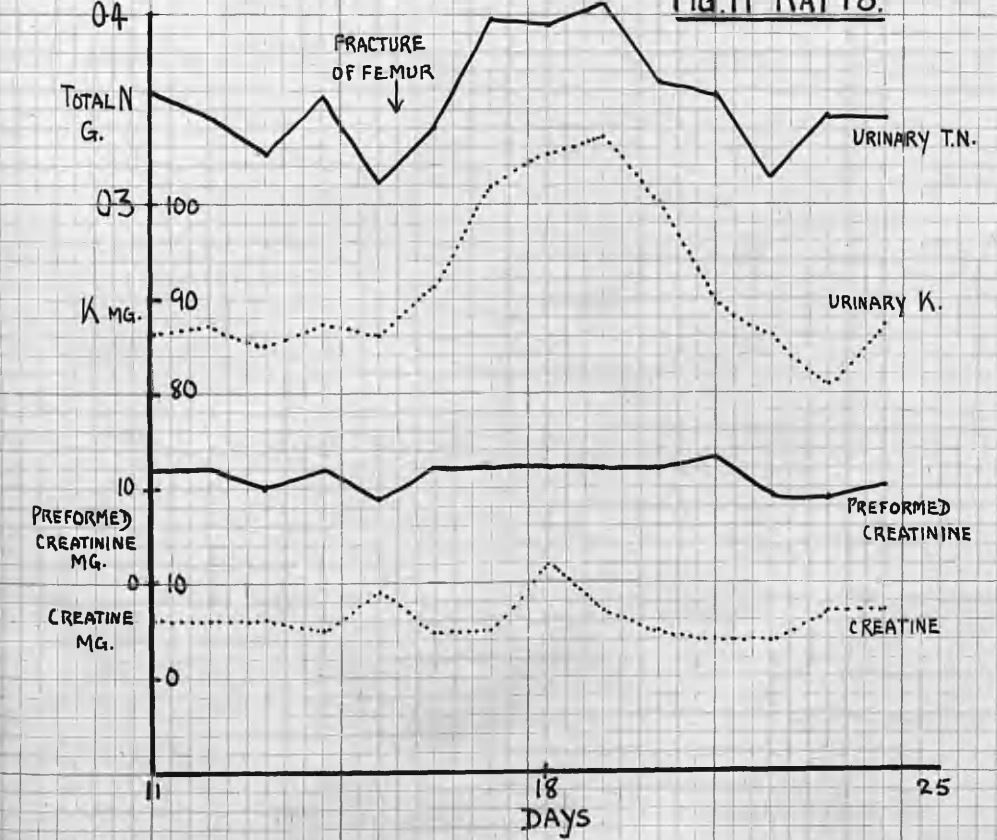


TABLE 13.

Rat 18. (Basal diet 17 g.)

Day of const. diet.	Body Wt. g.	Total N mg.	K mg.	Creatinine mg.	Creatine mg.
1	331				
11	331	359	86	12	6
12	-	346	87	12	6
13	331	326	85	10	6
14	-	367	87	12	5
15	337	311	86	9	9
Op.					
16	-	340	91	12	5
17	330	398	102	12	5
18	-	395	105	12	12
19	332	405	107	12	7
20	-	364	100	12	5
21		359	90	13	4
22		313	86	11	4
23		346	81	11	7
24	323	346	87	12	7

local anaesthesia is effective in allaying reflex muscle atrophy. The differences in weight between injured and uninjured limbs are so small and so irregular, that it is impossible to give a definite statement on this particular aspect of the problem.

In the case of Rat 17 the maximum excretion of K fell on the day following the N peak, the latter having taken place on the 3rd day following the trauma. This lag phenomenon was also noted in case of the human subject. The excess outputs of total N, creatine and K were 303 mg., 31 mg., and 69 mg. respectively. A small secondary rise in the excretion of N and creatine was noted on the 7th day after the injury.

The maximum N and K excretions in the case of Rat 18 were found on the 4th day following the operation, but only a very slight rise in the output of creatine was noted. The excess outputs of N and K were 188 mg. and 75 mg. respectively. Again a small secondary rise occurred in the N and K excretions, in this instance on the 9th day. In the human subject a similar secondary rise in N excretion was frequently noted.

In neither of these animals could the N and K losses be accounted for locally. In both animals the creatinine excretion remained very constant.

Third series: Rats 19 and 20 (Tables 14-15, Figs. 12-13).

These animals received a supplement of cane sugar in addition to the local anaesthetic. In the case of Rat 19 the carbohydrate could not be given before the 6th day following the operation for the animal had some difficulty in finishing its daily basal N-containing ration.

TABLE 14.

Rat 19. (Basal diet 16g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	K mg.	Na mg.	Creatinine mg.	Creatine mg.
1	Basal.	318					
3	"	-	311	81	-		
4	"	319	357	82	-		
5	"	-	362	78	-	11	5
6	"	321	307	80	-	9	7
7	"	-	312	85	383	11	4
8	"	328	316	82	389	10	6
Op.							
9	"	-	392	85	390	11	8
10	"	321	391	90	391	11	8
11	"	-	432	92	384	11	10
12	"	318	381	94	378	9	8
13	"	-	332	90	369	11	5
14	" + 5g.CHO	317	294	82	376	9	8
15	" + 3g.CHO	-	265	78	373	11	4
16	" " "	323	255	82	375	10	5
P.M.17	" " "	323.5	261	82	378	10	6

FIG. 12 RAT 19.

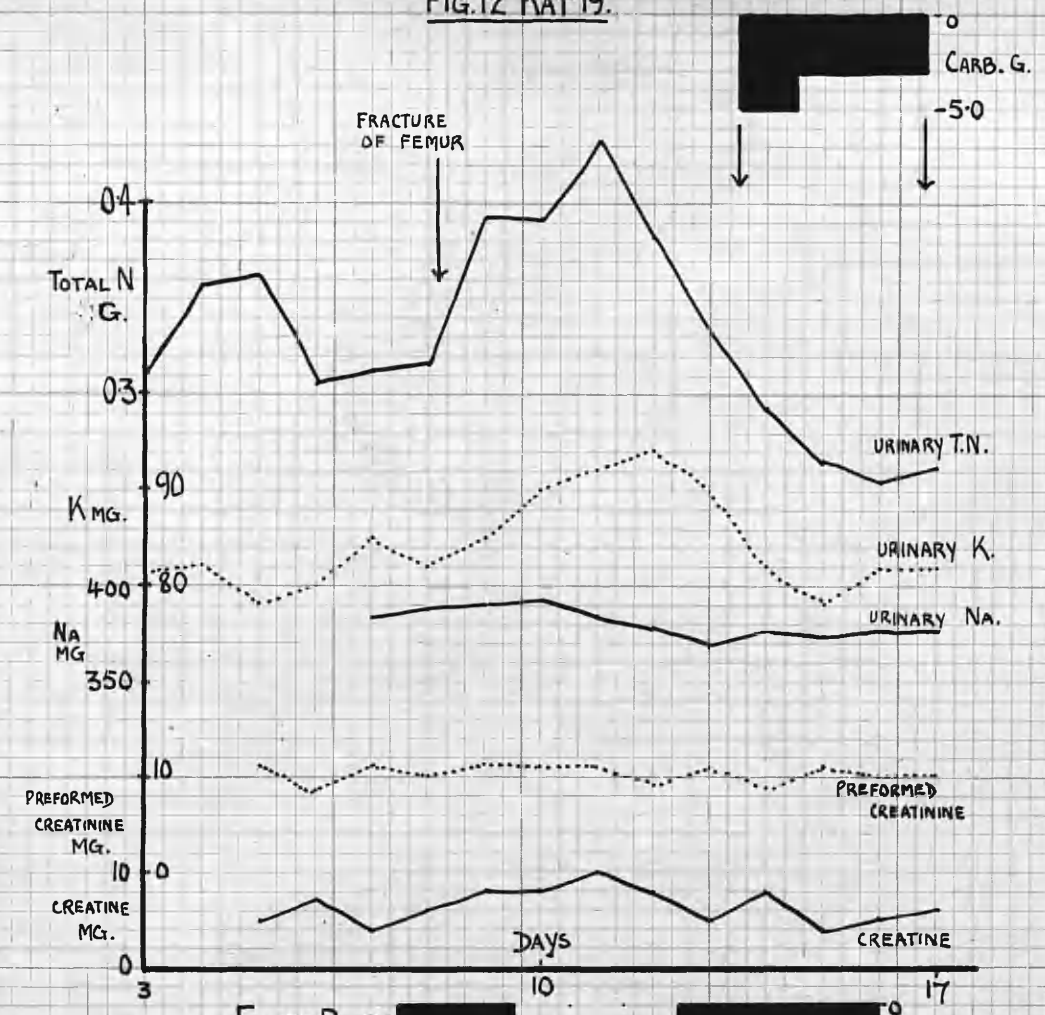


FIG. 13 RAT 20

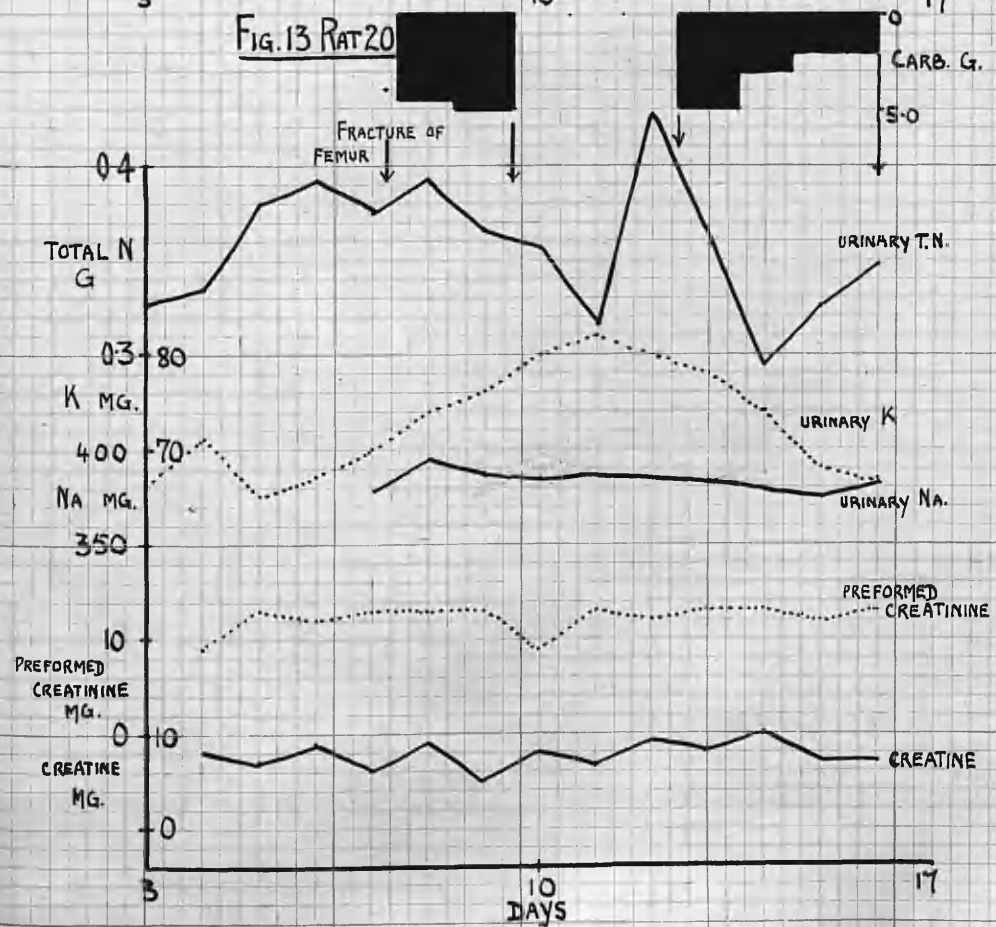


TABLE 15.

Rat 20. (Basal Diet 17 g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	K mg.	Na mg.	Creatinine mg.	Creatine mg.
1	Basal	336					
3	"	344	327	66	-		
4	"	-	334	71	-	9	8
5	"	345	380	65	-	13	7
6	"	-	392	67	-	12	9
7 Op.	"	-	374	70	379	13	6
8	" + 4.5g.CHO	-	392	74	395	13	9
9	" + 5 g. CHO	343	367	76	387	13	5
10	Basal	-	357	80	385	9	8
11	"	337	317	82	383	13	7
12	"	-	428	80	386	12	9
13	" + 5 g. CHO	338	362	78	384	13	8
14	" + 3 g. CHO	-	294	74	379	13	10
15	" + 2 g. CHO	346	326	68	377	12	7
P.M.16	" " "	348.5	349	67	382	13	7

The N and creatine maxima antedated that of K by a day and occurred on the 3rd day following the trauma. The administration of 5 g. cane sugar on the 6th day followed by 3 g. sugar on the days following, produced a definite fall in the N excretion without apparently affecting that of K. The Na excretion like that of creatinine remained relatively unchanged. The N, creatine and K losses amounted over the period of nine days to 60 mg., 12 mg. and 46 mg. respectively.

The sequence of events in the case of Rat 20 are best noted in Fig. 13. The interesting point is the difference in behaviour of the N and K. When graphed the general trend of the K values was similar to that seen in the previous experiments whereas N retention definitely took place. The gain in N was 138 mg., the loss of K 67 mg. The rise in creatine was practically negligible. This experiment demonstrates that the metabolic paths of these catabolites are not necessarily parallel. Again no change was noted in the levels of Na and creatinine.

Fourth series: Rats 21 and 22 (Tables 16-17, Figs. 14-15).

These two animals also received additional carbohydrate, but no local anaesthetic was injected. The great difficulty in these, and subsequent experiments involving ~~the~~ carbohydrate supplements, is to prevent failure of appetite for the basal N-containing diet. It is on this account that the supplements vary from day to day, for the animals' appetites were capricious.

The additional cane sugar to the diet of Rat 21 caused a definite depression in the N excretion, and the loss of K also appeared to be limited to some extent. The N and K losses over

TABLE 16.

Rat 21. (Basal Diet 15 g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	K mg.	Creatinine mg.	Creatine mg.
1	Basal	300				
3	"	294	357	80	-	-
4	"	-	360	81	-	-
5	"	296	347	73	11	6
6	"	-	330	76	9	6
7	"	295	338	81	10	5
8	"	-	343	78	10	8
9	"	300	352	75	9	7
10	"	300	336	76	9	7
Op.						
11	"	-	388	81	11	12
12	" + 5 g.CHO	288	324	84	9	7
13	Basal	299	445	86	10	7
14	" + 5g. CHO	-	337	81	10	9
15	" + 3g. CHO	299	312	77	11	4
16	" " "	-	265	74	10	3
17	" " "	299	285	76	11	5
18	" " "	-	273	78	10	6
P.M.19	" " "	303	275	76	10	5

FIG. 14 RAT 21

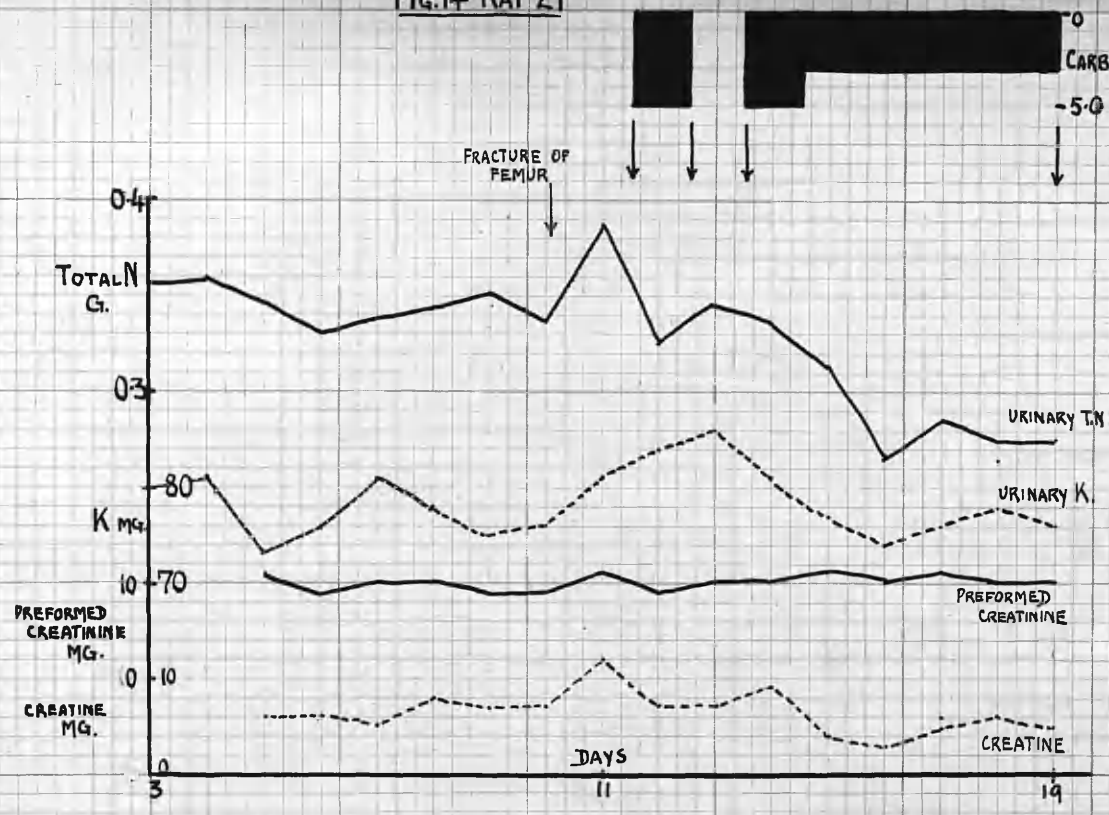


FIG. 15 RAT 22

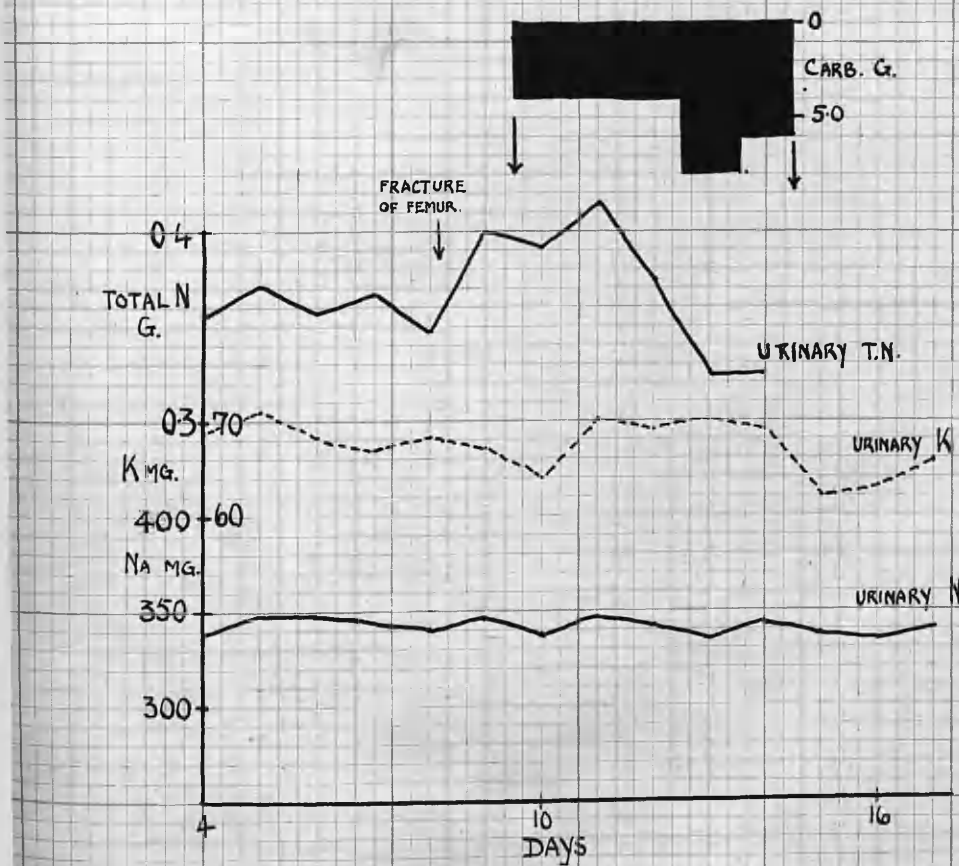


TABLE 17.

Rat 22. (Basal diet 18 g.)

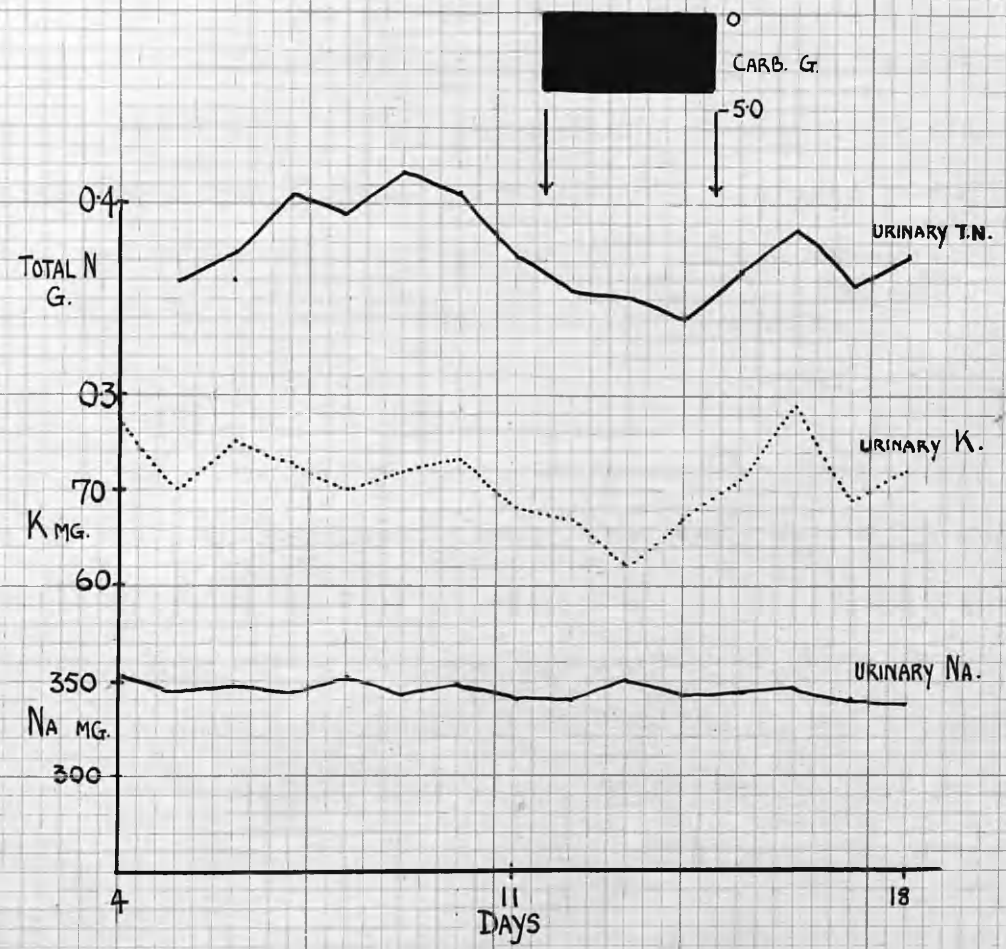
Day of diet.	Diet	Body Wt. g.	Total N mg.	K mg.	Na mg.
1	Basal	321			
4	"		357	69	339
5	"		371	71	348
6	"		357	68	348
7	"		366	67	345
8 Op.	"	312	348	68	340
9	"		400	67	346
10	" + 4g. CHO		392	64	339
11	" " "		414	70	347
12	" " "		373	69	341
13	" 8 g. "		325	70	335
14	" 6 g. "		325	69	343
15	Appetite failed.		T.N. not continued.	62	338
16	"			63	335
P.M. 17	"	319		66	340

TABLE 18.

Rat 23. (Basal diet 20g.)

Day of diet.	Diet	Body Wt. g.	Total N mg.	K mg.	Na mg.
1	Basal	374			
3	"			55	307
4	"			77	353
5	"		360	70	346
6	"		374	75	348
7	"		405	73	343
8	"		395	70	351
9	"		417	72	341
10	"		407	73	349
11	"	369	371	68	340
12	" + 4 g. CHO		354	67	340
13	" " "		351	62	350
14	" " "		340	67	342
15	Basal		365	71	343
16	"		387	79	345
17	"	373	357	69	340
18			372	72	338

FIG. 16 RAT 23.


0
CARB. G.
5·0
0·4
TOTAL N G.
URINARY T.N.
0·3
70
K MG.
URINARY K.
60
350
NA MG.
URINARY NA.
300
4
11
18
DAYS

the nine days were respectively 183 mg. and 20 mg. The creatine excretion appeared also to be limited in extent by this surplus feeding.

Up till the 6th day of the post operative period Rat 22 was able to consume the additional carbohydrate, appetite even for the basal diet failed thereafter. Curiously during this period there was virtually no loss of K over the six days though there was evidence of a rise and fall in its level. There was a loss of 69 mg. N in the course of the six days.

Fifth series: Rat 23 (Table 18, Fig. 16).

In view of the retention phenomena observed in the human subject when excess carbohydrate was administered, it was essential to determine if a similar result would occur in the rat. For this purpose a supplement of 4 g. cane sugar was given to an intact animal. A slight but definite depression in the urinary excretion of N and K but not Na took place, which was coupled with a gain in weight.

DISCUSSION BASED ON DATA DERIVED FROM SECOND GROUP OF RAT EXPERIMENTS.

Body and muscle weight changes: (Table 19).

In Rats 15-18 there were distinct losses of body weight and only a small fraction of this loss in weight could be accounted for by the diminution in the weight of the injured limb. It will be noted that the difference in weight between the normal and injured limbs was frequently smaller than the differences found to exist in the earlier series of three uninjured animals. This

TABLE 19.

Expt. No.	Nature of Experiment	Time interval between day of operation and post-mortem examination.	Loss in weight of animal g.	Loss in weight of injured limb ($\frac{1}{2}$ pelvis + limb). g.	Loss in weight of injured quadriceps femoris. g.	Total N loss over period. mg.	Creatine loss over period. mg.	K loss over period. mg.	N equivalent of weight lost by animal on basis of 3 g. N = 100 g. 'flesh'. mg.	N equivalent of weight lost by injured limb on basis of 3 g.N ≡ 100 g. 'flesh'. mg.
15	Open fracture - diet constant.	9	14	1.85	0.60	235 (8 days)	14 (8 days)	97 (8 days)	420	56
16	Open fracture - diet constant.	9	10	0.10	0.30	384 (9 days)	10 (9 days)	59 (9 days)	300	3
17	Open fracture + local anaesthetic - diet constant.	9	17.5	2.86	0.58	303 (9 days)	31 (9 days)	69 (9 days)	525	86
18	Open fracture + local anaesthetic - diet constant.	9	10	0.29	0.325	188 (9 days)	Nil	75 (9 days)	300	9
19	Open fracture + local anaesthetic + extra carbohydrate	9	4.5 (10 gm. in first 4 days.)	0.18	0.31	60 (9 days)	23 (9 days)	46 (9 days)	135	5
20	Open fracture + local anaesthetic + extra carbohydrate.	9	4.5 (gain)	1.12	0.41	138 (Gain 9 days)	negligible	67 (9 days)	135 (gain)	34
21	Open fracture + extra carbohydrate.	9	3 (gain)	0.70	0.38	183 (Gain 9 days)	6 (9 days)	20 (9 days)	90 (gain)	21
22	Open fracture + extra carbohydrate.	9	7 (gain)	0.53	0.42	69 (9 days)	-	Nil	210 (gain)	16

general lack of a marked difference may or may not be due to the increase in the vascular bed, and the other attendant reactions in the injured region such as fibrosis, etc., masking the loss in weight. The loss in weight of the quadriceps group was a much more constant effect.

Total urinary N.

In this group also, the marked loss of urinary N by the injured animals (15-18) was not accounted for by the loss in weight of the quadriceps femoris or by the wasting of the injured limb. On the other hand, if the N equivalent of the loss of body substance be calculated on the basis that the material lost is mainly muscle tissue in its entirety, the actual N loss is found to account for only approximately two thirds of the calculated N loss (Rat 16 was an exception to this, the actual N loss exceeding the calculated).

The administration of carbohydrate has two very similar effects: it may be that they are identical.

(1) It provides easily oxidisable material for the accelerated metabolic processes and so spares tissue protein and fat.

(2) By a simple plethora effect it causes protein retention in a manner analagous to that observed in the normal subject. This time the excessive supply of readily oxidisable material saves food protein and probably also food fat.

Creatine and creatinine.

The output of creatinine remained remarkably constant in all these experiments.

There was generally a slight creatinuria. The ratio of $\frac{\text{total N}}{\text{creatine}}$ was very much greater than that of muscle which is in the region of 6:1, indicating the conservation of creatine, a substance essential for the contractile activity of muscle. (The ratio in muscle is based on the total creatine value.) The phosphorus excretion of these animals was not determined. Experience of the excretion of phosphate by the injured human subject had already indicated that the output of this anion is relatively greater than that of creatine, for protein bound P is presumably liberated in the general catabolism.

The addition of extra carbohydrate tended to prevent the increase in creatinuria which was the normal concomitant of injury in these animals.

Urinary sodium and potassium.

Like that of creatinine the excretion of Na was quite unaffected by injury and by the additional carbohydrate. K on the other hand was eliminated in the urine to a relatively greater extent than even N. No constant relationship between the N and K excretions could be determined. The ratio $\frac{\text{total N}}{\text{K}}$ varied in Rats 15-18 from 2.5-8.3. The addition of carbohydrate diminished this K loss and in the intact animal even caused a retention of K. In Rats 20 and 21 who received extra sugar there was a loss of K and a gain in N. This discrepancy again emphasises that there

is no exact parallelism between the intermediary changes which lead to the liberation of N-containing metabolites and those which lead to the loss of the valuable base K.

At this point it is perhaps expedient to consider certain aspects of cellular metabolism.

The different tissues and fluids in the body exhibit considerable differences in their electrolyte constitution, yet the osmotic pressure of the total electrolytes is approximately uniform throughout. In the case of the human organism, in particular, it appears to be the rule that the principal base of the cells is K while that of the extracellular fluids is Na. The muscle system of the body will presumably then play a dominant rôle in the metabolism of K.

Katz (1896) found that human muscles had per 100 g. muscle tissue 79.9 mg. Na, 320 mg. K, 7.5 mg. Ca and 21.2 mg. Mg. The K value has been confirmed by numerous investigators. Although Katz did not analyse the muscles of the rat the values he found for the rabbit and cat are similar to those found by us in the case of the rat. The $\frac{N}{K}$ ratios for the quadriceps femoris of the normal and injured legs of a rat killed at the height of the catabolic disturbance as judged by the maximum urinary excretion were $\frac{3.349 g.}{0.387 g.}$ and $\frac{3.026}{0.303}$ g. i.e. 8.6:1 and 10:1 respectively. The diminutions in the N and K contents of the muscles were 9.6% and 21.8% respectively. The gastrocnemius of the normal and injured limbs of the same animal had ratios of respectively $\frac{3.056 g.}{0.366 g.}$ and $\frac{2.967}{0.342}$ g. the decrease in N and K contents

being 2.5% and 6.5%. These data though meagre, indicate that relative to N the K loss by muscle is the greater. Dr Watson, in this Institute, has furnished us with histochemical evidence of a definite loss of K from the injured muscles. Though there are certain indications that the K:Na ratio of the tissues may be influenced by the K:Na of the diet, very unnatural ratios have had to be used and their influence is of short duration (Miller, 1926, 1 and 2). In general, the organism strives to maintain a constant content of each base and this is presumably the optimum. Hence, it is all the more extraordinary that in the injured subject such a marked loss of K should occur.

The unequal distribution of K and Na throughout the body may depend on:

(1) the peculiar character of the cellular and intracellular media, or on

(2) differences in membrane permeability.

In starvation or wasting disease there occurs a loss of K paralleling the excretion of the other products which result from the wasting of the tissues. Gamble, Ross and Tisdall (1923) observed that during the first few days of fasting, there occurred a loss of Na, which was in excess of the amount contained in the autolysed tissue and was presumably due to the loss of extracellular fluids. After the first six days of a fifteen-day fast the K:Na ratio in the urine corresponded approximately to that in muscle tissue. In pneumonia a somewhat different picture is found, for during the pre-critical period Na and Cl are retained,

while K is eliminated in excess and may constitute 97% of the urinary base according to Salkowski (1871).

The processes at work in the injured subject differ from those in the fasting, in that there is not a parallel loss of Na, and in the fact that the K loss is relatively greater than that of N. In some ways the reaction is somewhat similar to that observed in fever, for a marked K loss is a dominant characteristic of the latter. The behaviour of Na is curious. A retention would have been anticipated as the result of injury.

This differential behaviour leads us to enquire how far it is justifiable to assume that the loss in weight is actually due to a complete disintegration of a small fraction of the muscle cells, accompanied by a partial, but parallel loss of the constituents of a large number of cells. K is spent more freely than N; creatine is preferentially retained and may in greater part be accounted for by the local tissue loss. N and K cannot therefore arise from a parallel loss of lesser or greater portions of the body's cells. What rôle, if any, is played by the liver in this strange phenomenon it is impossible to state. The greater loss of K must be due to a change in the character of the cellular and intra-cellular media and/or to an increased permeability of the cell membrane. In this connection, it is of interest that Andressen and Tammann (1933) found by their special technique an increase in the K and Ca content of the tissue fluids of aseptic wounds. Na and Cl diminished. During the hyperaemia which is one of the cardinal signs of the reaction to injury and which constitutes a phase of the healing process,

there is apparently an early increase in the permeability of the cells of the inflamed tissue (Schmidtmann and Matthes, 1927). This presumably causes a loss of K from the cytoplasm which would upset the iso-ionic balance of the tissue fluids and cause increased irritability of nerves. K injected into a wound does appear to promote hyperaemia (Schück, 1926: Häbler and Hummel, 1928) and causes pain lasting up to an hour after the injection.

The observations of Boyd (1898) and Greene (1919) on the biochemical changes in the muscle of the salmon during the fast of the spawning migration, have shown that there is little change in the structure of the muscle fibre. The augmentation in water content is only 6.6% (fat free basis). The mineral matter too remains remarkably constant. Muscle fat diminishes to the extent of 85% while the proteins are reduced to the extent of 30%. Since the muscles remain physiologically vigorous the conclusion was drawn that salmon can use, in the emergency of migration, stored muscle protein without jeopardizing the protoplasmic integrity of the muscle. The work of Moulton (1920) on the muscle-fibres of the underfed steer are also of interest. Underfeeding decreased the weight of lean flesh by about 44.8 kilos. in an animal weighing about 300 kg. The loss of protein, calculated from the N content, was 10.9 kg. He found little difference in the diameter of muscle fibres of very fat or thin steers. On the other hand, the muscle fibres of a very thin steer had only one fifth as great a cross section as that of a thin steer and one sixth as great as that of a fat steer. The length of the segments of the muscle fibres showed a similar decrease.

No evidence was found of a disintegration of muscle fibres. The very thin fibre still functioned as a muscle fibre. The change must have been due to a resorption of inter- and intra-cellular substances.

In this consideration of muscle wasting, it is perhaps of interest to refer to the recent work of Westenbrink and Krabbe (1936), which has shown that when a denervated gastrocnemius is analysed some three weeks after section, the normal percentage composition of the muscle is preserved with but three exceptions, despite a reduction in weight to less than one half of its former self. The exceptions are creatine phosphate, free orthophosphoric acid and myosin. There is a relative decrease of from 20-30% in these components. Here we have a further example of an absence of a parallelism between the loss of the constituents of muscle tissue during a wasting process.

CONCLUSIONS.

In the light of these and other experiments on the rat and human subject, it can be stated that as a consequence of a severe injury such as the fracture of a long bone, a dislocation, an effusion into a joint, a laceration of the soft tissues or an incision into a knee joint, there is a marked disturbance of general as well as local metabolism. This is characterised by a rise in the basal consumption of oxygen, by slight fever (rise of 1-2°C) and by tachycardia, signs indicative of an increased metabolism. These changes are generally paralleled by a marked loss of total N, total S, total P, K and to less extent creatine in the urine. Na and creatinine remain relatively unaltered, occasionally a slight fall is noted. These products reach their maximum elimination about the third to fourth day in the case of the rat and about the sixth day in man, though with the latter the actual time is more variable. There is frequently a secondary small rise in these catabolites several days later.

Trauma, such as has been described, also causes an immediate and marked disturbance of the total and relative proportions of the plasma proteins, in the shape of a slight fall in the albumin moiety coupled with a marked rise in the globulin fraction. Fibrinogen is also frequently considerably raised.

There was no definite correlation between the apparent damage and the metabolic disturbance. This is mainly due to

the fact that it is impossible to assess accurately the actual damage done.

The fact that additional non-protein food, in excess of the ordinary requirements, spares to a considerable extent this loss of body substance, indicates that an increased demand for oxidisable material for energy purposes is one of the principal factors. In the human subject diets of high calorie value (up to 5000 Cal.) and of very high first class protein content failed to prevent a negative N-balance at the height of the disturbance. This suggests that part of the rise in the urinary excretion of these catabolites is probably caused by autolytic processes. These are presumably localised to the site of the injury. The protein sparing effect of carbohydrate was much more striking in the case of the rat. It is probable that if the urine collections in the case of the rat had been made at shorter intervals a similar experience to that found in the human subject would have been observed. A day in the life of a rat is relatively a much longer period of time than a day in the life of man.

Following the peak of the metabolic disturbance the various processes declined in intensity and in those cases receiving adequate nourishment N, etc. retention occurred. Manipulations of the injured part in the early stages of the healing process, particularly if surgical, appeared to produce a further disturbance of the metabolic processes as characterised by an increased N elimination and change in the level of the plasma proteins.

The excessive loss of K is probably the result of changes in cell permeability which are intimately related to the healing process. There is evidence to show that the muscles adjacent to the fracture lose relatively more K than N. The K loss is, however, greater than can be accounted for by local changes. This does not hold for creatine. The creatinuria is probably related to processes taking place in the muscles of the injured area. These processes are in part probably autolytic, in part due to reflex wasting.

The increased metabolic processes are more general than local and presumably also involve loss of body carbohydrate and fat. Since certain of them can be allayed to a considerable extent by raising the food intake, it would appear to be good clinical practice to feed injured subjects with the maximum intake of energy providing material which they are capable of ingesting. In addition there are several indications for maintaining a high protein diet during the convalescent period. The fact that injured subjects do not generally exhibit such voracious appetites as their apparent requirements would lead us to expect, may be an indication to exercise caution when advocating forced feeding. In our experience accident cases can generally tolerate, and sometimes enjoy, such high protein and high calorie diets, for a period at least. Appetite generally fails later. This is in accord with our experience of the normal subject both rat and human.

Disuse atrophy, though a contributory factor, does not form an adequate explanation of this strange phenomenon, as the

loss of body substance is greatly in excess of that produced by experimental disuse (Cuthbertson, 1930). It has long been known that muscular wasting, associated with bone or joint disease, is much more rapid and extreme than that which occurs with simple disuse of a limb. It is also true that there is a more rapid and extreme wasting in limbs immobilised for some inflammatory or traumatic lesion than in limbs immobilised to correct a deformity. The reason for this lies not in the degree of immobilisation but in a reflex trophic effect affecting certain groups of muscles more than others in the same region. Such reflex atrophy is dependent on the integrity of the afferent nerve paths (Harding, 1925, 1926 and 1929).

One is tempted to speculate that herein may lie an explanation for this trophic change; the resultant rapid wasting of muscle and utilisation of the body's reserves of carbohydrate, fat and protein being the response to the organism's urgent demand for material for maintenance and repair. As was suggested in an earlier paper (Cuthbertson, 1930), injury to an animal generally leads to lessened activity and a lessened activity to a diminished food supply. The necessity is urgent and the body may require, and may on occasion even prefer, to catabolise its reserves to meet the exigencies of the moment. These experiments indicate that the wasting of the injured and adjacent groups of muscles is not appreciably mitigated by a high calorie diet. The local loss of substance probably proceeds unabated by such dietary measures. It is only the excessive demands for potential energy which can be met by providing additional food. Energy is

required to effect the various stages in the healing process, but we are left wondering why the apparent expenditure is in excess of the apparent need.

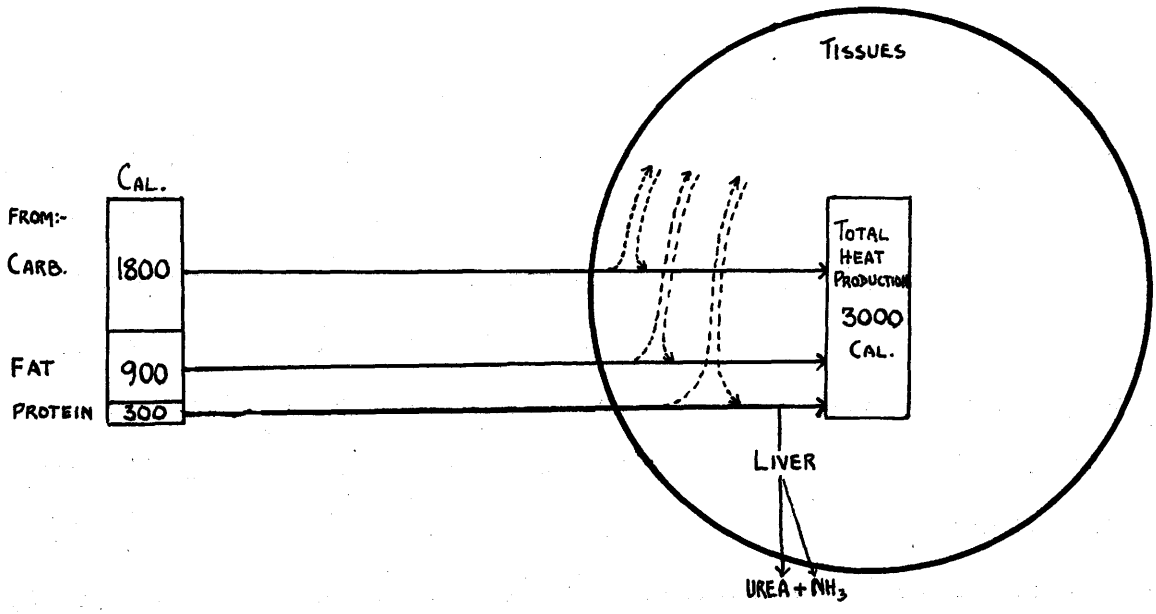
An attempt will now be made to harmonise these varied observations on protein metabolism under one general scheme. A diagrammatic method of presentation has been adopted. It must be said at the outset that this scheme is very crude and merely outlines what the writer believes to be the metabolic fate of protein, carbohydrate and fat in the body, without defining the intricacies of the intermediary metabolism.

In each diagram the left hand rectangle represents the potential energy of the absorbed products of the digestion of protein, carbohydrate and fat. The rectangle on the right represents the total heat production in the tissues, the latter being portrayed by the area within the circle.

For convenience, the net energy of the ordinary intake is taken as 3000 Cal., made up of 1800 Cal. carbohydrate, 900 Cal. fat and 300 Cal. protein. The percentage distribution of 60, 30 and 10 per cent respectively for these principles probably lies very near that of the average for the mass of the people living in this country, and may even be the optimum for people living under such climatic conditions as exist in these islands. There is a certain degree of correlation between the absolute and relative amounts of these three proximate principles and the

degree of latitude, for the further north the greater is the consumption of protein and fat, and the further south the greater the relative intake of carbohydrate. Undoubtedly availability and purchasing power play a not inconsiderable part in the selection of diet, particularly as regards fat, but climatic conditions both directly and indirectly probably play the dominant rôle. The relatively high first class protein intake of the Masai, an African native race, is certainly an exception to this somewhat dogmatic statement. Probably there are other exceptions to be found, which are based on racial or religious traditions.

C and N equilibrium.

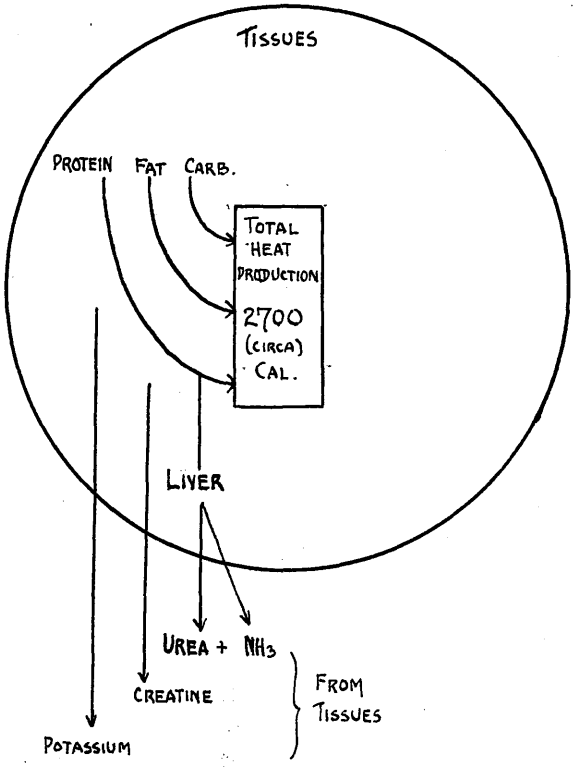


It is assumed that material with a fuel value equal to the calorie value of the absorbed nutrients is burnt, and that the bulk of this material is derived more or less directly from the food. All the evidence points to the existence of a lag period in the case of protein. At least this lag period is appreciable when the total calorie intake is altered or the protein moiety alone. This lag period probably represents the time taken for those amino acids which have not been selected in the first instance for deaminization by the liver, to reach the tissue fluids, and either there or in the cells themselves, be constituted into, or be added to, the proteins characteristic of the fluid or particular tissue cell. Following on this, there will occur a slow liberation of those amino acids not required by the tissues. These amino acids will be deaminized and utilised as a source of energy along with the great bulk of the other deaminized amino acids. In addition to the constant metabolism of the tissue cells, a variable amount of readily oxidisable material must be available to meet the changing energy requirements of these cells. For this purpose we presume that there is a preferential choice of carbohydrate, presumably mainly glucose. In addition fat is burnt. The deaminized residues of the amino acids are reckoned as inferior in quality to carbohydrate as a source of fuel, but that a certain moiety is always used in the combustion processes.

Starvation: Physical work output reckoned same as in previous example.

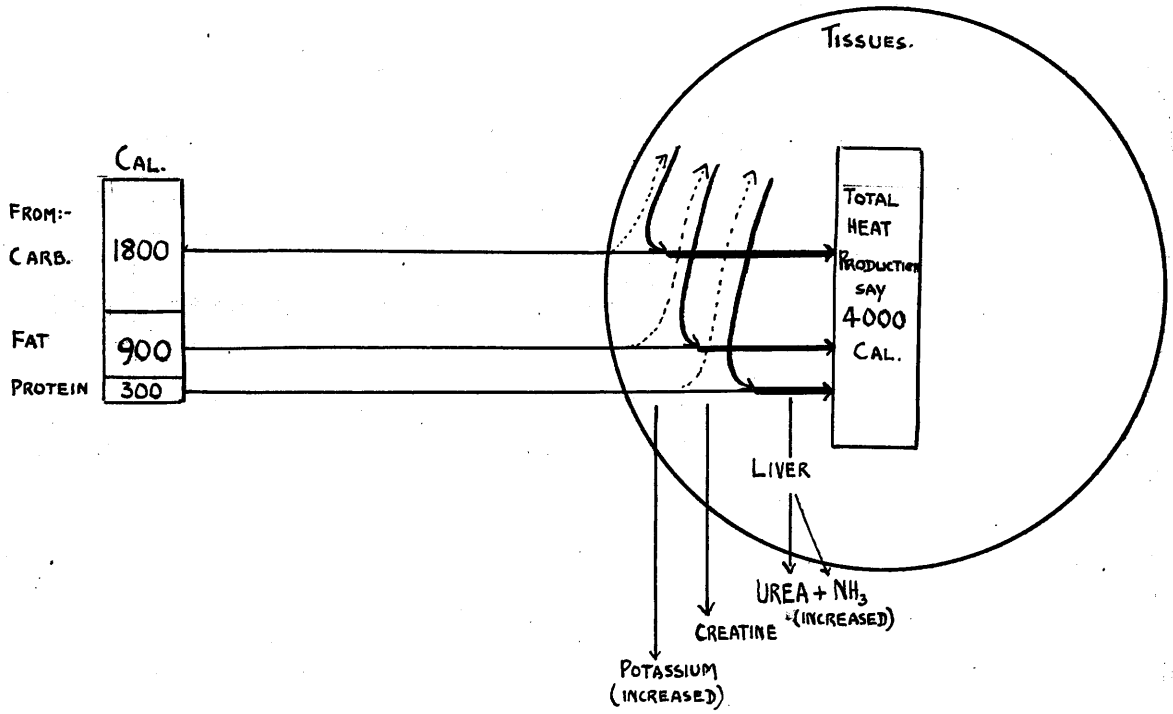
CAL. INTAKE

NIL.



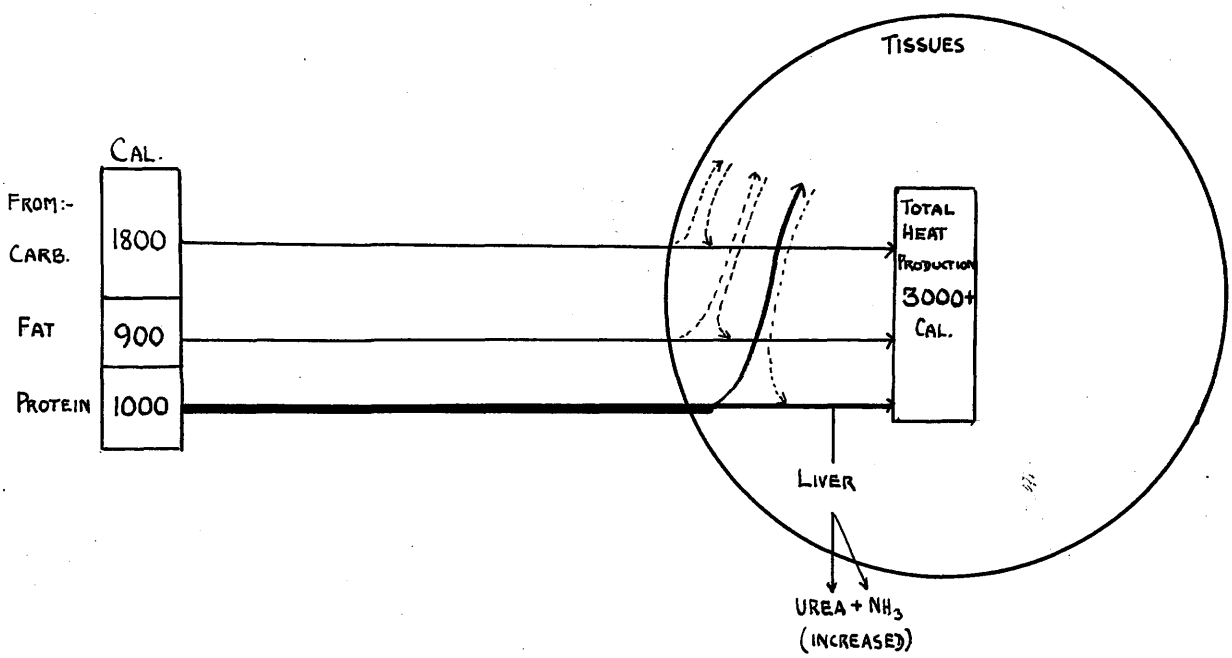
In starvation the fuel consumption is smaller, for the body probably acts more economically when the source of its combustible material is itself. The carbohydrate stores are soon exhausted and then the fat and finally the protein stores are called upon.

Severe injury or febrile wasting disease.



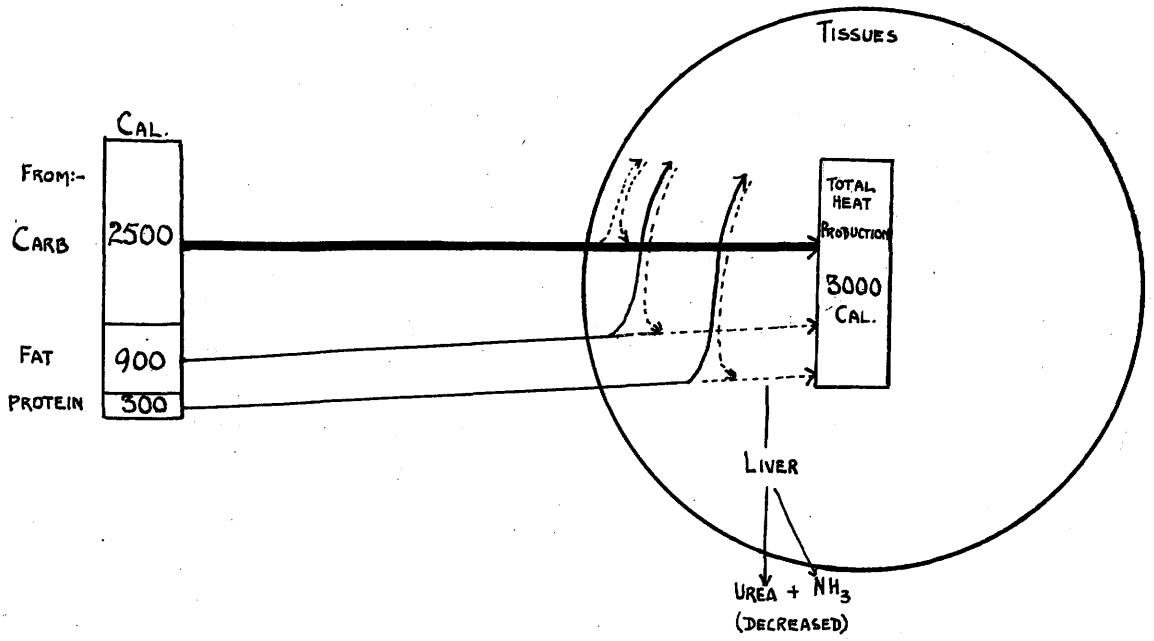
In this instance the activities of the body demand a higher metabolic rate. To meet the increased demand for readily available fuel the body cashes its reserves. If the calorie value of the intake of food can be made commensurate with the metabolic requirements, this breakdown of the tissue reserves may be stopped or at least masked by an opposite effect.

Ordinary diet + surplus protein.



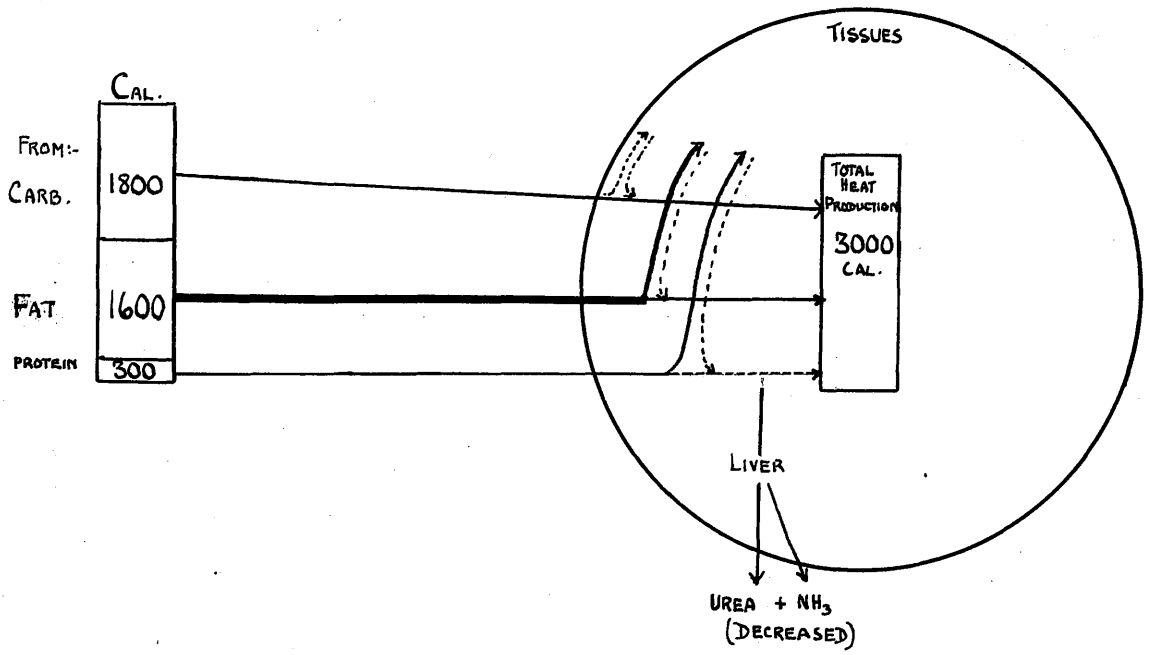
For comparative purposes the additional intake of protein is taken as equivalent to 700 Cal. The total heat production is slightly enhanced and again carbohydrate is preferentially burned. As all the protein and fat cannot be burned without raising the energy output, something must remain unburnt and be retained. It is believed that part of the amino acids and perhaps also part of the fat, is retained in the organism: the fat in the definite storage depots, the amino acids contributing to the mass of the existing tissue proteins.

Ordinary diet + surplus carbohydrate.



The additional intake of carbohydrate is assumed to be 700 Cal. Carbohydrate is preferentially burned as the storage capacity for this principle is very limited, if it be not turned into fat or protein. There is always a certain amount of protein and fat utilised, for the combustible mixture is derived from all three proximate principles. Since 2500 out of the total 3000 Cal. are obtainable from the food carbohydrate the excess calories must be stored in the form of protein and fat. The reduction in T.N. output is not sufficient to indicate that all the food N is stored, only a certain fraction is retained within the tissues. Similarly too with fat. The reduction in N output bears a relationship to the magnitude of the excess calorie intake.

Ordinary diet + surplus fat.



The additional fat intake is taken as equivalent to 700 Cal. Carbohydrate is again preferentially burned. As the capacity for fat storage is considerable, it is possible to dispose of a fraction of the excess energy intake in this way. Owing to the plethora of fat all the protein is not burned and storage of a fraction of the ingested N takes place. In this case the N-saving effect is less marked than with carbohydrate, owing to the fact that the storage of fat as fat can take place more readily than the transformation of carbohydrate into fat and then its storage as such.

The writer considers that this scheme offers a reasonable description of the various phenomena observed, but as Terence has put it "Nullum est jam dictum quod non dictum sit prius".

SUMMARY.

1. The ingestion of diets very rich in first class protein and of high calorie value, by persons suffering from the fracture of one or more of their long bones as the result of direct violence, modified considerably the marked loss of body protein which normally occurs under such circumstances. At the height of the catabolic disturbance, however, such diets still failed to prevent this loss of protein.

2. The addition of extra carbohydrate to rats which were in receipt of a fractured femur, apparently exercised a sparing effect on tissue protein, which was more marked than that observed in the human subject. The reason for this probably lay in the fact, that a day in the life of a rat is relatively a much longer period of time, than a day in the life of man. It is possible that had analyses been made at more frequent intervals than once every 24 hours, results similar to those found in the human subject might have been obtained.

3. Measures such as massage and manipulation, the addition of meat extractives, glycine, hydrolysate of mixed ox tissue, gelatin and sodium caseinate, and diets of high calorie value but average protein content similarly failed to stem the loss of protein, and generally proved less successful in mitigating the drain on the body's reserves.

4. The catabolic disturbance is characterised by an increase in the basal consumption of oxygen with attendant rise in pulse rate and temperature, and by parallel rises in the urinary output of total N, total S, total P and to a less extent K. Na excretion remains relatively unchanged.

5. The creatinuria which develops and parallels the rise in total N is accompanied by little change in the creatinine excretion; such change as occurred took the form of a slight diminution during the period of maximum creatinuria.

6. If it be assumed that muscle tissue is mainly contributing to this excessive urinary excretion, then the loss of K is relatively greater than the loss of N, and the loss of creatine is relatively smaller than the N loss.

7. Analyses of the quadriceps femoris overlying the fractured femur revealed that there was relatively a greater loss of K than N. The S:N ratio of the excess S and N outputs in the urine was 1:16.36 - a value close to that of muscle.

8. Owing to the fact that the change in weight of the injured limb was relatively insignificant in comparison with the variability of weight found to exist amongst the dissected hind quarters of intact animals, it was impossible to determine whether the introduction of a local anaesthetic with a presumed prolonged action allayed reflex wasting.

9. The loss in weight of the quadriceps femoris of the injured limb was not prevented by forced feeding. This was only to be expected.

10. If it be presumed that the excess N comes from muscle then the loss of muscle substance cannot account for more than two thirds of the total loss of body weight. The presumption is that the reserves of carbohydrate and fat are also called on to meet the demand for readily oxidisable material.

11. Trauma such as has been described also causes, at least in the human subject, an immediate and marked disturbance of the plasma proteins. Both the total amount and relative proportions are affected. The general effect is a slight fall in the albumin moiety coupled with a very definite rise in the globulin fraction. Fibrinogen was often found to be appreciably raised.

12. Subsequent manipulations of the injured part were found to cause a considerable increase in the globulin and fibrinogen fractions, and an increased loss of N was also found in the urine.

In conclusion, I wish to express my thanks to Professor E. P. Cathcart for his constant encouragement and useful criticism during the course of this work, and to Professor G. M. Wishart for his helpful co-operation at all times. My thanks are also due Professor J. Shaw Dunn and all my colleagues who have aided me at various stages of this work. Further, I am indebted to Sister Cumming and her nursing staff for their unremitting care of my patients.

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